ENDOCRINOLOGY

VOLUME 36 MARCH, 1945 NUMBER 3

THE OVARIAN HORMONES AND SPONTANEOUS RUNNING ACTIVITY IN THE FEMALE RAT[1]

WILLIAM C. YOUNG[2] AND WILLIAM R. FISH

From the Yerkes Laboratories of Primate Biology and Yale University School of Medicine

ORANGE PARK, FLORIDA AND NEW HAVEN, CONNECTICUT

THE PATTERN of behavior which is shown by the white rat at the time of estrus is composed of the copulatory response given on stimulation by the male (Stone, 1922; Hemmingsen, 1933; Ball, 1937; Blandau, Boling and Young, 1941), often a male-like mounting activity (Long and Evans, 1922; Hemmingsen, 1933; Beach, 1938; Beach and Rasquin, 1942; Ball, 1940), and a greatly increased running activity (Wang, 1923; Slonaker, 1924; Hemmingsen, 1933). The latter behavior has been induced in spayed females by treatment with a variety of naturally occurring estrogens and synthetic substances having estrogenic properties (Bugbee and Simond, 1926; Wang, cited by Richter, 1927; Hemmingsen, 1933; Richter, 1934; Richter and Hartman, 1934; Hemmingsen and Krarup, 1937) and, although the amount of activity following treatment did not always equal that shown prior to ovariectomy (Bugbee and Simond, 1926; Slonaker, 1927; Hemingsen, 1933; Hemmingsen and Krarup, 1937), the opinion is held generally that the running activity associated with estrus is stimulated by the ovarian estrogens or their metabolites.

Partly because replacement therapies in which estrogens were used were not always effective and partly because the appearance of the mating responses in the rat seems to depend on the action of progesterone as well as estrogen (Boling and Blandau, 1939; Beach, 1942a),

Received for publication August 4, 1944.

[1] This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

[2] Now at Cedar Crest College, Allentown, Pennsylvania.

Copyright, 1945, by the Association for the Study of Internal Secretions.

we became interested in ascertaining if progesterone has any role in the production of running activity. As our experiments progressed, it became evident that such is not the case; the administration of an estrogen was found to be sufficient and progesterone, in the quantities injected, appeared to be without effect. In addition, a further difference was found: Whereas a single treatment with estrogen and progesterone is sufficient to induce normal mating responses, the maintenance of a constant source of estrogenic stimulation seems important for the amount of running activity which can be induced. Discovery of these differences between running activity and the mating responses suggested that more factors are involved in the production of the total pattern of estrous behavior than we have hitherto supposed and it is the experiments leading to this conclusion that are summarized and discussed in the sections which follow.

MATERIAL AND METHODS

Data were obtained from 42 rats which were members of a vigorous strain that had been maintained for several years. The age of the animals when they were placed in the revolving cages varied from 71 to 150 days; the age at the end of the experimental period varied from 107 to 413 days. The number of revolutions per day was recorded from the time the animals were placed in the cages, but the amount of activity during the preoperative periods as shown in table 1 is that displayed after intervals from 8 to 23 days had elapsed. Following determination of the level of activity characteristic of each animal, ovariectomy was performed and treatment was begun either immediately or within 28 days, depending on the purpose of the experiment.

The nature of the hormonal treatment varied. A first group of 12 animals was injected subcutaneously with different quantities of estradiol benzoate[3] or with 10 R.U. estradiol benzoate followed 48 hours later with 0.4 I.U. progesterone. A second group of 20 animals was injected with different quantities of aqueous estrone[4] and a third group of 14 animals received 2.8 to 3.7 mg. pellets of estrone implanted subcutaneously.

The 12 revolving drum cages which were constructed as a unit did not differ in any essential detail from those described by Wang (1923) and Hemmingsen (1933). The circumference of each wheel was 1 meter. The animals in the cages were kept in a heated room in the Medical School where day to night fluctuations in temperature were small.

Readings were taken once a day at 4 P.M. Food was always available. When a single injection was given, the four readings beginning 24 or 48 hours after the injection were used in determining the amount of activity per day following such treatment. When a series of injections was given, the readings that were used were those made 24 or 48 hours after the first injection and daily thereafter throughout the period treatment was given.

[3] Estradiol benzoate (Progynon-B) and progesterone (Proluton) were made available through the courtesy of Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

[4] Aqueous estrone (Theelin) and pellets of estrone were furnished through the courtesy of Dr. Oliver Kamm of Parke, Davis and Company, Detroit, Michigan.

When pellets were implanted, the readings that were used were those taken 24 or 96 hours after the operation and daily thereafter for the balance of the experimental period. The per cent change as shown in the tables was calculated from the amount of activity prior to ovariectomy. All figures have been rounded off to the nearer whole number.

In order to ascertain if any relationship existed between the ovarian condition and the amount of activity, the ovaries from 12 animals showing a variable amount of running activity were preserved, sectioned serially and studied by Dr. John L. Boling. The pellets of estrone were implanted into 10 animals by Dr. James G. Wilson as a check on the earlier results obtained from 4 animals. Many of the readings were taken by Mrs. Harriet C. Wilson. To these colleagues, the writers are deeply grateful.

EXPERIMENTAL

The data are summarized in table 1. It can be seen that neither estradiol benzoate in the quantities given nor estradiol benzoate

TABLE 1. AVERAGE REVOLUTIONS PER DAY PRIOR TO OVARIECTOMY AND FOLLOWING THE ADMINISTRATION OF ESTRADIOL BENZOATE, ESTRADIOL BENZOATE IN COMBINATION WITH PROGESTERONE, AND ESTRONE

	Number of animals	Revolutions during preoperative period	Revolutions during experimental period	Per cent change
Spayed	12	6051 ± 1202	922 ± 379	−85 ± 4
10 R.U. estradiol benzoate, first injections	12	6051 ± 1202	2123 ± 597	−66 ± 5
10 R.U. estradiol benzoate, second injections	12	6051 ± 1202	375 ± 117	−92 ± 2
40 R.U. estradiol benzoate, first injections	12	6051 ± 1202	403 ± 126	−92 ± 2
40 R.U. estradiol benzoate, second injections	12	6051 ± 1202	2548 ± 827	−53 + 19
10 R.U. estradiol benzoate + 0.4 I.U. progesterone, first injections	12	6051 ± 1202	949 ± 284	−82 ± 4
10 R.U. estradiol benzoate + 0.4 I.U. progesterone, second injections	12	6051 ± 1202	1675 ± 554	−69 ± 12
10 I.U. aqueous estrone daily for 14 days	12	9671 ± 1018	7982 ± 904	−16 ± 6
Estrone pellets implanted at time of ovariectomy	10	4340 ± 689	5254 ± 845	+25 ± 10
Estrone pellets implanted 28 days after ovariectomy	4	4751 ± 561	4746 ± 1168	0 ± 12

followed by progesterone was effective in restoring running activity to the level shown prior to ovariectomy. Aqueous estrone given daily was more effective than estradiol benzoate, but our most successful experiments were those in which pellets of estrone were implanted either at the time of ovariectomy or 4 weeks later and from which an estimated 33 I.U. to 101 I.U. per day were absorbed (Forbes, 1942, 1943). The conclusion to be drawn from these results is that running activity, in contrast to the other parts of the estrous behavior pattern, is stimulated by continuous treatment with estrone rather than by single injections of estradiol benzoate alone or in combination with progesterone.

XUM

The importance of a continuous supply of hormone for the maintenance of running activity seems clearly indicated. The restoration of activity to 53% of that displayed during the preoperative period by the second injection of 40 R.U. estradiol benzoate is attributed to the circumstance that frequent injections of 10 R.U. of this hormone had been given during the 6-week period prior to the final treatment with estradiol benzoate. In another experiment injections of 20 I.U. and 40 I.U. estrone made once each 4-day period for 32 days restored activity in four animals to an average of only 58% of that displayed prior to ovariectomy. Finally, the success achieved by Richter (1934)

TABLE 2. THE DAILY RUNNING ACTIVITY IN REVOLUTIONS PER DAY OF 24 ANIMALS BEFORE OVARIECTOMY AND DURING THE EXPERIMENTAL PERIOD. THE ANIMALS ARE LISTED IN THE DESCENDING ORDER OF ACTIVITY BEFORE OVARIECTOMY

Animal number	During 40 days before spaying	During 4 days, 15 days after spaying	During 4 days following 10 R.U. estradiol benzoate	During 4 days following 10 R.U. estradiol benzoate +0.4 I.U. progesterone	Animal number	During 12 days before spaying	During 12 days treatment with 10 I.U. aqueous estrone daily
2	13,003	4,732	7,027	3,003	13	16,219	14,633
12	11,858	1,986	3,205	1,457	19	13,500	10,033
3	9,473	1,768	4,838	1,919	18	12,460	8,760
11	9,052	906	3,686	2,489	22	11,860	8,679
1	7,578	156	1,411	520	21	11,569	5,206
5	7,106	471	2,051	34	17	9,126	6,544
10	5,904	19	699	237	23	9,104	10,366
7	4,061	100	1,084	494	15	8,691	10,099
8	1,661	224	353	230	16	8,211	6,312
9	1,505	529	774	834	14	6,971	8,079
4	1,052	60	133	41	20	5,498	5,193
6	356	111	211	129	24	2,848	1,874

and Richter and Hartman (1934) may perhaps also have been due to the fact that the hormone was continuously present in the drinking water or was given daily.

The activity that was shown following replacement therapy was generally proportional to the amount shown prior to ovariectomy regardless of the treatment (table 2). Hoskins and Bevin (1940) noted this relationship in their study of the effect of estriol glucuronide on the running activity of senile male rats, and a similar relationship between the character of the response prior to ovariectomy and after the hormonal treatment of spayed animals was noted when the mating responses of guinea pigs (Boling, Young and Dempsey, 1938) and rats (Boling, Blandau, Rundlett and Young, 1941) were being studied.

At the time of the observations on the guinea pig, the differences in the responsiveness of individual animals were attributed to differences in the sensitivity of the tissues mediating estrous behavior to stimulation by estrogenic hormones (Young, Dempsey, Myers and Hagquist, 1938). Beach (1942b) in his analysis of factors involved in

the arousal, maintenance and manifestation of sexual excitement in male rats likewise emphasizes the importance of the character of the soma for the behavior which is shown, as does Koster (1943) who produced an alteration of the sensory-motor system in female rats by the injection of testosterone propionate. In this study the facts 1) that the ovaries from rats 1 to 12 (table 2) were normal and 2) that neither structural differences nor marked differences in the number of active corpora lutea were seen in the ovaries removed from the most active and from the least active animals, are taken to indicate that unidentified somatic or at least extra-ovarian factors do much to ac-

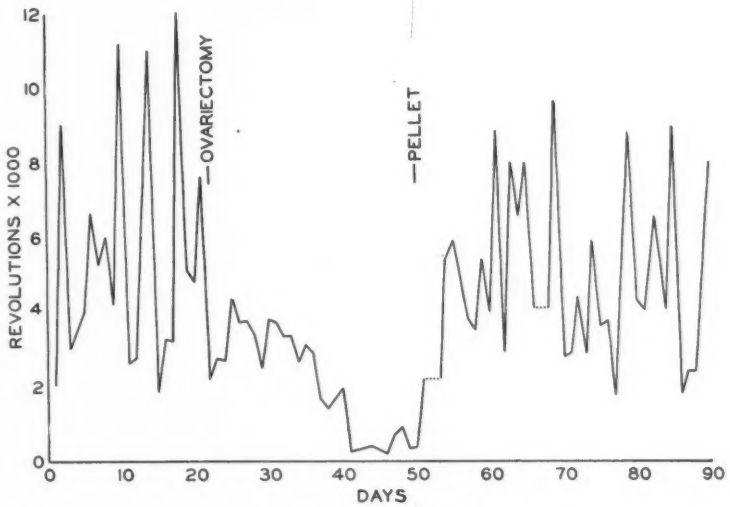


FIG. 1. Running activity of animal 54 prior to ovariectomy, during an interval without treatment and following implantation of a 3.0 mg. pellet of estrone.

count for differences in the amount of running activity. Twelve corpora lutea were found in the ovaries from the most active animal and 10 were present in the ovaries from the least active animal. Other evidence in support of this hypothesis comes from the observations by Heller (1932) that the accessory reproductive organs of inactive male rats were normal, thus indicating that testis-hormone was being produced.

Not evident from the tables is the day to day variation in activity displayed by animals injected daily with aqueous estrone and by those in which pellets of estrone were implanted. That this variation was nearly as great as that displayed by intact animals is clear from fig. 1 in which the curve of activity for animal 54 is given for the preoperative period, after ovariectomy and prior to pellet implantation

and during the period when the pellet of estrone was the source of estrogenic stimulation. Similar variations occurred in the records of most of the other animals in this group, indeed there is no animal in which activity was maintained at a constant level.

DISCUSSION

The use of a procedure by means of which running activity can be produced in the spayed rat in an amount which approximates that displayed prior to ovariectomy has enabled us to obtain information that can be used in a comparison of the mating response with running activity. Certain similarities are clearly evident: 1) The character of running activity and of the mating responses in treated spayed individuals bears a close resemblance to that shown during the preoperative period. 2) Animals that were inactive or failed to come into heat before ovariectomy usually did not give a strong response to quantities of administered hormones that were adequate for animals in which the estrous reactions were more nearly normal. 3) Neither the length of estrus nor the amount of running activity was related to the number of maturing follicles.

The differences are equally definite and they are of such a nature that the physiological mechanism which mediates the production of running activity is thought to be quite unlike that mediating the mating responses: 1) Progesterone does not appear to be involved in the production of running activity. 2) Running activity was maintained at a high level in spayed rats only when a more or less constant source of estrone was maintained, but a single injection of estrogen when followed by progesterone is sufficient for the induction of a normal mating response in most animals (Boling and Blandau, 1939).

The apparent need of a constant source of estrogenic substance, if a complete restoration of running activity is to be achieved, is puzzling in view of the cyclic nature of ovarian changes and of running activity in the intact animal. In addition, the great variation in the amount of daily running activity in animals having a constant source of estrogenic stimulation, with some indication that it is cyclic, cannot be accounted for. Kostitch and Télébakovitch (1928), del Castillo and Calatroni (1930) and Zuckerman (1938, 1941) have suggested that a basic rhythm exists in the threshold or sensitivity to estrogenic stimulation, but the possibility is one that should be investigated further.

The results from the present investigation are not new in the sense that a procedure which is effective in stimulating normal running activity in ovariectomized animals has for the first time been developed. The addition of small quantities of pregnancy urine to the drinking water (Richter, 1934) and the daily injection of estrone (Richter and Hartman, 1934) had previously been found to be effective. Interest in the data presented here lies rather in what is suggested

with respect to the factors which act as determining influences on the total pattern of estrous behavior in the intact, untreated animal. If our interpretation based on observations of the rat and guinea pig is correct, the mating response, the male-like mounting behavior and running activity are stimulated by the cyclically produced secretions of the ovaries rather than by a single substance. But in addition to the factor of a multiplicity of hormones having a selectivity or specificity of action, the situation is complicated by the circumstance that there is not a common threshold or sensitivity for the three parts of the pattern. Many observations suggest that each of the mechanisms mediating the three parts of the total pattern responds in its own manner and that the behavior which an animal displays is more strongly influenced by the sensitivity of these mechanisms to hormonal substances than by any quantitative fluctuations in the amount of secreted substances, provided the threshold of stimulation has been reached. Mating responses without male-like mounting behavior are seen in the rat and guinea pig (Long and Evans, 1922; Hemmingsen, 1933; Young, Dempsey and Myers, 1935; Beach, 1938; Ball, 1940), mounting behavior has been recorded in the absence of heat (Young, Dempsey and Myers, 1935), and Hemmingsen (1933) and Hemmingsen and Krarup (1937) state that running activity is sometimes displayed when mating responses cannot be elicited. The result is about what one would expect. Variation in the pattern is great even when ovarian function is regular and, as far as we can tell, maintained at about the same quantitative level. Furthermore, variations in one part of the pattern seem to occur more or less independently of variations in the other parts with the consequence that no two animals show the same pattern of behavior. On the other hand, Rundquist's (1933) observation that the per cent of fertile matings tends to become lower, that the number of days together before littering increases, and that the average number of offspring decreases must not be lost sight of. The implication that some correlation exists between running activity and other phases of the reproductive process would appear to be reasonable and should be used as a point of departure for further studies.

Quite apart, however, from the extent of correlation in the degree to which the parts of the estrous behavior pattern are manifested, the factor or factors which determine or modify the responsiveness of the tissues are of basic importance for the behavior which is shown. Such factors as seasonal influences, the nutritional level, and age of the animals are discussed by Young (1941) in a review of the subject as it stood at that time. Since then Beach (1942b) and Koster (1943) have directed attention to the importance of the excitatory effectiveness of the stimulus-object for the responsiveness of the rat, and Rundquist (1933) and Brody (1942) have suggested that some of the variation in running activity may be genetically determined. Under experimental

XUM

conditions, sensitivity to the action of the gonadal hormones has been modified in males and females by treatment with estrogens and androgens (Wilson, Young and Hamilton, 1940; Wilson, Hamilton and Young, 1941; Wilson, 1943; Wilson and Wilson, 1943). Apparent desensitization to estrogens has been accomplished by the placement of hypothalamic lesions (Brookhart, Dey and Ranson, 1941), but such a procedure caused an interruption of neural pathways rather than any change in sensitivity. The entire subject of responsiveness to hormonal action is in need of thorough study and, possessing as we do the techniques whereby it is impossible to induce the behavior characteristic of individual rats and guinea pigs, it should be only a matter of time until a considerable progress will have been achieved.

SUMMARY

The complete restoration of running activity in the ovariectomized albino rat was accomplished with rather small quantities of estrone administered in such a way that a constant source of the hormone was maintained. Estradiol benzoate in the quantities injected was less effective and supplementary injections of progesterone were completely dispensable. It is not implied that estrone is the only estrogenic substance having this action.

The amount of activity following replacement therapy was generally proportional to the amount shown prior to ovariectomy rather than to the amount of hormone given. This fact, when taken in conjunction with the observation that the ovaries of the less active animals were not different from those of the more active animals, has led us to postulate that the character of running activity like that of the mating responses is strongly influenced by unidentified somatic factors, or at least extra-ovarian factors, rather than by quantitative fluctuations in the amount of available estrogenic substances.

Attention is called to a number of fundamental similarities and differences between running activity and the mating response. The suggestion is made that these two features of the estrous behavior pattern are mediated by different mechanisms.

REFERENCES

Ball, J.: *Comp. Psychol. Monog.* 14: no. 1. 1937.
Ball, J.: *J. Comp. Psychol.* 29: 151. 1940.
Beach, F. A.: *J. Genet. Psychol.* 53: 329. 1938.
Beach, F. A.: *Proc. Soc. Exper. Biol. & Med.* 51: 369. 1942, a.
Beach, F. A.: *Psychosom. Med.* 4: 173. 1942, b.
Beach, F. A., and P. Rasquin: *Endocrinoogy* 31: 393. 1942.
Blandau, R. J., J. L. Boling and W. C. Young: *Anat. Rec.* 79: 453. 1941.
Boling, J. L., and R. J. Blandau: *Endocrinology.* 25: 359. 1939.
Boling, J. L., W. C. Young and E. W. Dempsey: *Endocrinology.* 23: 182. 1938.
Boling, J. L., R. J. Blandau, B. Rundlett and W. C. Young: *Anat. Rec.* 80: 155. 1941.
Brody, E. G.: *Comp. Psychol. Monog.* 17: no. 5, 1942.
Brookhart, J. M., F. L. Dey and S. W. Ranson: *Endocrinology.* 28: 561. 1941.

BUGBEE, E. P., AND A. E. SIMOND: *Endocrinology.* 10: 349. 1926.
DEL CASTILLO, E. B., AND C. J. CALATRONI: *Compt. rend. Soc. de Biol.* 104: 1024. 1930.
FORBES, T. R.: *Endocrinology.* 30: 761. 1942.
FORBES, T. R.: *Endocrinology.* 32: 282. 1943.
HELLER, R. E.: *Endocrinology.* 16: 626. 1932.
HEMMINGSEN, A. M.: *Skandinav. Arch. f. Physiol.* 65: 97. 1933.
HEMMINGSEN, A. M., AND N. B. KRARAUP: *Det. Kgl. Danske Videns. Selskab. Biol. Med.* 13: no. 8. 1937.
HOSKINS, R. G., AND S. BEVIN: *Endocrinology.* 26: 829. 1940.
KOSTER, R.: *Endocrinology.* 33: 337. 1943.
KOSTITCH, A., AND A. TÉLÉBAKOVITCH: *Compt. rend. Soc. de biol.* 100: 51. 1928.
LONG, J. A., AND H. M. EVANS: *Memoirs Univ. Calif.* 6: 1. 1922.
RICHTER, C. P.: *Quart. Rev. Biol.* 2: 307. 1927.
RICHTER, C. P.: *Am. J. Physiol.* 110: 499. 1934.
RICHTER, C. P., AND C. G. HARTMAN: *Am. J. Physiol.* 108: 136. 1934.
RUNDQUIST, E. A.: *J. Comp. Psychol.* 16: 415. 1933.
SLONAKER, J. R.: *Am. J. Physiol.* 68: 294. 1924.
SLONAKER, J. R.: *Am. J. Physiol.* 81: 325. 1927.
STONE, C. P.: *J. Comp. Psychol.* 2: 95. 1922.
WANG, G. H.: *Comp. Psychol. Monog.* 2: no. 6, 1923.
WILSON, J. G.: *Anat. Rec.* 86: 341. 1943.
WILSON, J. G., W. C. YOUNG AND J. B. HAMILTON: *Yale J. Biol. & Med.* 13: 189. 1940.
WILSON, J. G., J. B. HAMILTON AND W. C. YOUNG: *Endocrinology.* 29: 784. 1941.
WILSON, J. G., AND H. C. WILSON: *Endocrinology.* 33: 353. 1943.
YOUNG, W. C.: *Quart. Rev. Biol.* 16: 135, 311. 1941.
YOUNG, W. C., E. W. DEMPSEY AND H. I. MYERS: *J. Comp. Psychol.* 19: 313. 1935.
YOUNG, W. C., E. W. DEMPSEY, H. I. MYERS AND C. W. HAGQUIST: *Am. J. Anat.* 63: 457. 1938.
ZUCKERMAN, S.: *Actualités Sci. et Indust.* No. 602: 121. 1938.
ZUCKERMAN, S.: *J. Endocrinol.* 2: 263. 1941.

XUM

THE GONADOTROPIC ACTIVITY OF THE ANTERIOR PITUITARY OF COCKERELS[1]

W. R. BRENEMAN

From the Waterman Institute and Department of Zoology,[2]
Indiana University

BLOOMINGTON, INDIANA

THE DETERMINATION of the gonadotropic activity of the anterior pituitary in the chick has been handicapped until recently because relatively large amounts of avian glandular substance were thought to be necessary to elicit responses in test animals. Recently, however, Jaap (1935), Meyer, Mellish, and Kupperman (1939), Phillips (1942) and Riley and Fraaps (1942), have developed techniques which make it possible to test individual glands. A sensitive test was reported in the two latter papers in which the growth of the vaginal epithelium of rat and mouse was utilized. This technique demonstrated that it was feasible to make a more detailed physiological study of the pituitary of the chick during the first few months after hatching.

The time from hatching to 90 days of age is a period of great physiological change in White Leghorns as is evidenced by the fact that the anterior pituitary weight increases more than 9 times and the testicular weight more than 80 times during this interval, Breneman (1944). These results suggested that pituitary secretion is initiated perhaps as early as the 5th and certainly by the 15th day post hatching. Payne (1942) demonstrated that the basophilic cells of the anterior lobe are developing rapidly and are apparently active during this period. The cytological evidence, therefore, tends to confirm the hypothesis of early pituitary activity.

An analysis of the data for pituitary weights showed that the growth rate is not uniform but that there is a definite plateau from 30 to 50 days after hatching, Breneman (1944). The growth curve for the gonads, to the contrary, does not have such a plateau but growth, which begins immediately after hatching, is especially marked after the 30 to 35 day period. It is the intent of this paper to estimate the gonadotrophic activity of the anterior pituitary glands of cockerels during the first three months of life and to correlate these estimations with the testis growth during the same period.

Received for publication October 19, 1944.

[1] Supported in part by a grant from the American Philosophical Society.

[2] Contribution No. 337 from the Department of Zoology and No. 94 from the Waterman Institute, Indiana University.

MATERIALS AND METHODS

The determination of the gonadotrophic activity of the anterior-pituitary was made by assaying the effect of the glands on testicular weight in chicks. The method employed was that described by Byerly and Burrows (1938), in which the chicks received no food nor water during the 96-hour assay period. All animals were kept in the shipping boxes which contained four compartments, each holding 25 chicks. All injections were made subcutaneously and, with one exception, were given in 5 divided dosages of 1/5 cc. each and the total amount of pituitary gland administered was equivalent to 5 mg. of fresh anterior lobe. The testes and body weights of the birds were taken at autopsy and the gonad weights were averaged both as a percent body weight and as an actual weight average. The standard errors for the two did not differ significantly but only the percentage weights are presented in this paper because these probably express better the hormonal influence of the anterior pituitary on gonad growth.

The estimation of pituitary potency was made in terms of "*chick units.*" It was noted that a 25% increase in gonad weight above the control average is significant if 10 or more birds were used in an essay, therefore a slight additional allowance for error, namely a 35% increase using ten or more birds, was considered to constitute a "*chick unit*" (C.U.) and all the estimations of activity shown in Graphs I and II were determined on this basis. The chicks used in all experiments were single comb White Leghorns and were secured from one hatchery.[3] Only birds received within 12 hours after hatching were used in the pituitary assay series.

The use by other investigators of the rat and mouse for assay of fowl pituitary, suggested that a similar test should be made of our material for purposes of comparison. Mice were injected, therefore, in a manner similar to the chicks, but were not starved. Body, uterine, and ovarian weights were taken at autopsy. The vagina was examined to see if it was patent and was fixed in Bouins for examination of the epithelium. The mice were twenty-three days old at the beginning of treatment and were autopsied on the twenty-sixth day.[4]

The pituitary glands were collected from birds of known ages by a method which had been described in a previous paper, Breneman (1944). Since the pituitary material was injected in the chick series in amounts equivalent to 5 mg. of fresh tissue, the potency could be determined both per milligram and per gland. This makes it possible to compare unit weight activity and total activity of glands for the different age groups and in addition establishes a normal series for future comparison with pituitaries from experimental animals.

RESULTS AND DISCUSSION

Acetone-dried pituitaries: One of the major problems in the experiments was to have the assay animals available when the cockerels were at the proper age. It had been hoped to assay fresh pituitaries at 5-day intervals, but it was impossible to secure the assay animals for each period. This can be observed in Graph I in which such varied

[3] Martin's Ramsey Hatchery, Ramsey, Indiana.
[4] Harlan Small Animal Industries, Cumberland, Indiana.

XUM

times as 54, 63 and 72 days may be noted. The use of acetone-dried pituitaries offered a possible solution to the difficulty, but Kupperman, Elder and Meyer (1941) reported that acetone drying resulted in a decrease of about 50% in the gonadotrophic potency of chicken pituitaries. Table I, however, demonstrates that acetone-dried glands can be successfully used in the chick assays.

It is immediately evident from Table I that, using the chick test, the acetone-dried pituitaries are just as effective as are the fresh glands. Actually, the acetone-dried glands elicited better responses in all five examples shown, although the differences between the means are not significant. This raises a fundamental question concerning the divergence in our results from those reported by Kupperman, Elder and Meyer. The fresh glands were weighed and placed in acetone

TABLE I. RESPONSE OF CHICKS TO INJECTION OF ACETONE-DRIED AND FRESH ANTERIOR-PITUITARY

Chicks received 5 mg. equivalent of pituitary gland in 5 divided doses. Chick Unit (C.U.) represents a 35% increase over the control gonad weight.

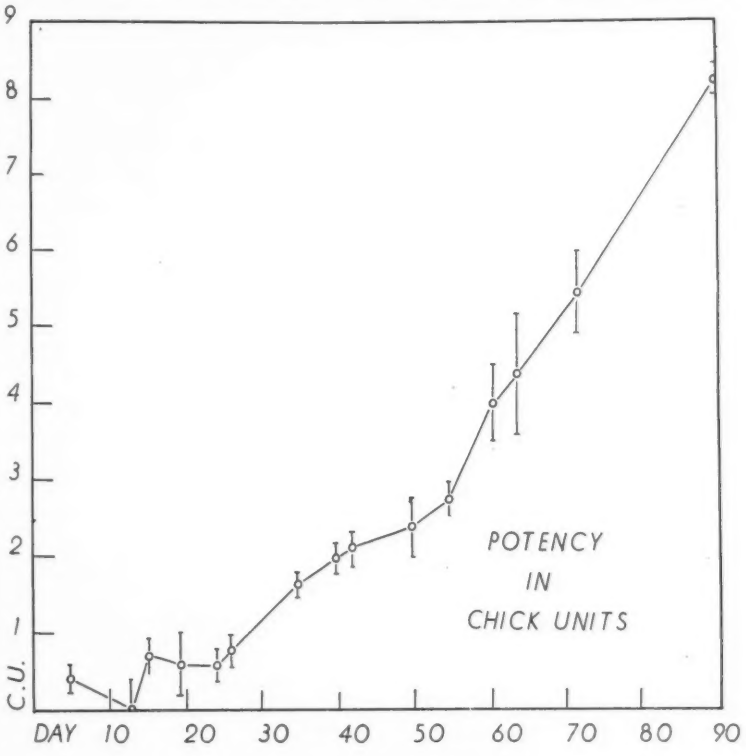
Acetone-Dried Pituitaries			Fresh Pituitaries		
Age in days	Experimental animals	C.U. δ_m	Age	Experimental animals	C.U. δ_m
19	8[1]	0.8–0.16	19	12	0.4–0.26
42	16	2.1–0.22	42	32	1.9–0.21
54	13	2.8–0.16	54	10	2.6–0.54
64	12	4.5–0.37	64	15	4.3–0.86
72	12	5.1–0.63	72	12	5.0–0.71

Each experimental animal received a dosage equivalent to a total of 5 mg. of fresh pituitary gland.

[1] This is the only experimental series in which less than 10 birds were used in the determination of "chick units."

within a very few minutes after killing and were stored in acetone until ready for use, at which time they were quickly dried, weighed, ground fine and taken up in water for injection. The chief difference in this technique and that of Kupperman et al, was that after changing acetone several times, the latter stored the pituitaries in a dry state. They were able to show, however, that air-dried pituitaries were 100% effective after 80 days storage and the differences in storage techniques, therefore, appear to be of doubtful significance. A second alternative, the difference in the test animal used, may be a more important factor. It will be observed in table II that acetone-dried pituitaries of 20-day-old chicks were not effective in mice, but, in table I, that 19-day-old pituitaries produced good responses in the chicks. Is it possible that the gonadotrophic complex may be modified in such a fashion by acetone drying that the mouse ovaries do respond as well as the chick testes? The fact that in the chick test the same species (and variety) of donor and assay animal is used may be an important contributing factor in the excellence of the responses.

Pituitary assay-chicks: The chick assay data for the pituitaries of different ages are presented in Graph I. At least one control group was run for each point on the graph because, in spite of the fact that the control averages were remarkably uniform, a few groups varied. Specific examples of this can be cited for two experiments in which the control means were 6.1 mg. and 4.6 mg. The determination of the



GRAPH I. Potency of cockerel pituitary in chick units—C.U. Vertical lines represent standard errors. A total of 864 pituitaries were used to determine the curve.

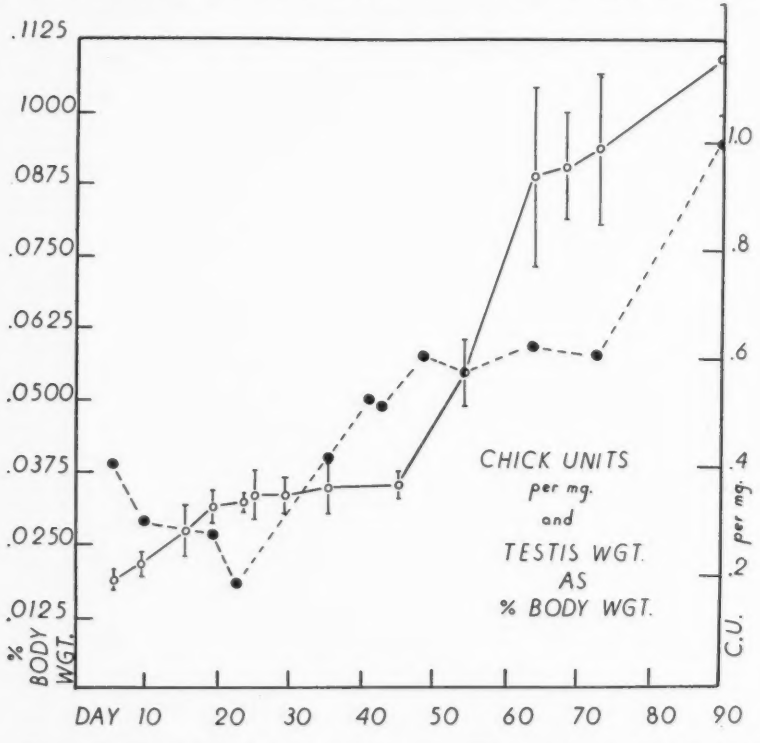
potency in terms of "chick units" was, therefore, taken as an increment over the controls run for each experiment.

The range in the potency of the glands is from 0.5 C.U. at day 5 to 8.3 C.U. at day 90. After the 23rd day, the rise in potency is very uniform and closely approximates a straight-line response. Previous to the 20th day, there is a significant amount of gonad stimulating activity in the glands but the total potency increases only slightly. If the standard errors are considered, it is possible that the curve is essentially a plateau at this period. This observation would again support the data previously reported, Breneman (1940), and Payne (1942),

XUM

that the activity of the pituitary begins early and is markedly increased after the 15th day.

Some appreciation of the extensive physiological changes which occur in the pituitary can be better gained by reference to the potency of the glands per milligram of tissue for the various periods, which is shown in Graph II. There is a steady decrease in the activity per milligram from the 5th to the 23rd day. The curve rises sharply from the



GRAPH II. The growth of the testes and the per milligram potency of the pituitaries in terms of chick units—C.U. Solid line represents testicular weights and the dotted line represents pituitary potency in C.U. Vertical lines are standard errors. A total of 530 pairs of testes were used to determine the curve for testicular weight.

23rd to the 49th day, exceeding even the slope of the "total-activity" curve in this respect, but then plateaus until the 73rd day when it again abruptly swings upward, The least amount of activity is found on the 23rd day, 0.19 C.U. per milligram; and the maximum, 1.00 C.U. per milligram on the 90th day.

These results are interesting when compared with those reported for the rat by Clark (1935). She noted that the female pituitaries were much more potent during the 13th to 20th day interval than

they were even at maturity and this greater activity was even more striking on a per unit weight basis. The male pituitaries, however, showed a more gradual increase in potency. The pituitary of the male chick would, therefore, differ greatly from that of the rat with respect to relative potency at different ages. The only similarity would be in the greater potency per milligram in the very young animal and this involves the comparison of the cockerel with the female rat.

It was noted in the account of pituitary growth, Breneman (1944), that from hatching to 30 days of age is a period of rapid weight increase but from 30 to 50 days there is a plateau in the growth curve following which the weight again increases in the latter part of the curve. Two possible explanations for potency variation per unit of weight are immediately suggested: first that there is a differential growth of certain cell types within the pituitary; or second that there is not a uniform rate of secretion by the cells during all periods and that this variation in secretion rate is influenced by the secretion of androgen by the testes.

An analysis of the first point can be made in part on the basis of the observations by Payne on the growth and secretory activity of the cells of the chick pituitary. He has described a rather uniform development of the basophilic cells during about two-thirds of the period we have been analyzing. The chromophobes likewise increase in prominence in the gland at the same time and, therefore, the acidophilic cells are relatively less conspicuous. If both acidophilic and basophilic cells contribute to the gonad stimulating ability of the pituitary, the increase in the number of chromophobes would act as a depressing factor in hormone production by displacing an equivalent number of acidophiles in a given area of gland. Should only the basophiles be concerned with the production of the gonadotrophin we are testing, then we would expect to find a uniform increase in the rate of hormone production. Neither of these explanations, therefore, would seem to account for the erratic fluctuations in potency which we observe.

The growth of the testes is primarily controlled by the total output of gonadotrophin from the pituitary and androgens in turn also will influence the output of hormone by the anterior lobe of the chick, Breneman (1940). This interaction between these glands affords the second possible explanation for the fluctuations in the potency per milligram of the pituitary, and may be analyzed by reference in Graph II to the variations in testicular weight expressed as a percentage of body weight. This presentation of the data was used because the percentages reflect primarily that portion of total gonad growth which is under pituitary control.

The testes increase rather uniformly in weight from the 5th to the 23rd day, the net increment being approximately 170%. During this same interval the net decrease in the milligram potency of the pitui-

XUM

tary is approximately 120%. There is a definite plateau in the relative growth of the gonads during the next 20 days but it is during this interval that the pituitary potency greatly increases and then strikes another plateau from the 49th to the 73rd days. Here again the testicular data are striking because after day 45 the percentage weights rise abruptly but show a tendency to level off again at about the 65th day. It is at the 73rd day that the pituitary potency curve rises and the general correlation between the two sets of data is maintained, namely: as the testes increase in size the slope of the milligram potency curve is downward or shows a plateau but when the testis curve plateaus the potency curve rises.

This striking relationship suggests, of course, an intimate interrelationship between the pituitary and testes in early development. The growth of the comb throughout these periods gives ample evidence for a considerable secretion of male hormone by the testes and if it could be assumed that male hormone secretion was proportional to testis size, the hypothesis might be advanced that as the androgen output increases the pituitary secretion is suppressed. Subsequently, however, the decrease in gonadotrophic hormone would be accompanied by a lowered stimulation of the gonads, a decreased androgen output and, finally, a decrease in the inhibition of the pituitary. Thus it would be possible to explain the alternating plateaus and increases, and a few days "lag" between the potency curve and the testes curve such as observed at the 45–50 day and 68–73 day periods would be expected.

The proof for such a hypothesis, however, must be in the analysis of the hormone output of the testes during the critical periods and likewise on a more detailed study of the effects of the androgens on pituitary secretion. We are making such a study at the present time.

It is interesting, in conclusion, to compare these results for the cockerel with somewhat similar studies by Riddle (1931) and Riddle and Schooley (1935) for the ringdove and pigeon. It was shown in these forms that the testes did not grow appreciably until after two or more months of age and that the gonadotrophic hormone secretion is not evident until the same period has been reached. Since growth and secretion begin almost immediately after hatching in both pituitary and gonad of the chick, these observations clearly indicate that generalizations concerning avian pituitary-gonad relationships should be advanced very cautiously.

Riddle suggested that the inactivity of the pituitary in the first two months probably explains the excellence of the ringdove as a test animal for follicle stimulating hormone because the assay picture is not complicated by the presence of endogenous gonadotrophin. Riddle and Schooley were able to get excellent responses with as little as 38 mg. of pituitary gland substance. The fact that the chick responds even better than the dove or pigeon to pituitary hormone, namely to

5 milligrams of gland, is not necessarily a contradiction of Riddle's explanation concerning the excellence of the ringdove response, because the chick test is most effective during a period of inanition when anterior pituitary secretion must be negligible.

Pituitary assay—mice: Various groups of chick pituitaries were assayed on mice using both the uterine weight increase and differentiation on the vaginal epithelium for assay. A representative selection of such experiments utilizing uterine weights of 191 mice are shown in Table II. It is obvious that the mouse responds well to small

TABLE II. MOUSE UTERINE WEIGHTS IN MGS.

23 day old female mice received 5 injections of pituitary suspension in 3 day period. Autopsied on 26th day.

Treatment	No.	Av. δ_m
Control	39	5.7 ± 0.27
.25 mg. acetone-dried pituitary of 20-day cockerel	11	5.6 ± 0.35
.50 mg. acetone-dried pituitary of 20-day cockerel	12	5.8 ± 0.30
1.00 mg. acetone-dried pituitary of 20-day cockerel	10	5.9 ± 0.35
1–60 day cockerel pituitary	9	12.6 ± 1.27
2–60 day cockerel pituitaries	9	12.8 ± 1.25
3–60 day cockerel pituitaries	6	13.7 ± 1.68
1–60 day pullet pituitary	6	9.3 ± 0.48
2–60 day pullet pituitaries	6	9.0 ± 0.20
1–70 day cockerel pituitary	9	14.3 ± 2.10
2–70 day cockerel pituitaries	10	29.2 ± 2.27
3–70 day cockerel pituitaries	9	31.1 ± 2.41
1–90 day cockerel pituitary	9	11.2 ± 0.82
2–90 day cockerel pituitaries	6	14.5 ± 2.00
3–90 day cockerel pituitaries	6	15.0 ± 1.49
1–40 day capon pituitary	8	7.1 ± 0.50
2–40 day capon pituitaries	6	9.7 ± 0.75
½–70 day capon pituitary	12	11.4 ± 0.74
1–70 day capon pituitary	8	16.3 ± 2.29

amounts of chick anterior pituitary substance. Unfortunately the data leave much to be desired as regards quantitative measurements as is well illustrated in the groups which were given glands from 60 day old cockerels and pullets.

Definite increases in uterine weights are indicated in the majority of the series but there are no significant differences within the series given 1, 2 or 3 male pituitaries nor within the groups which received 1 or 2 pullet pituitaries. The uteri of mice which were given 70 day cockerel pituitaries exceed the average for the 60 day group and there is a good qualitative difference between those which were injected with one and two pituitaries in the former group, but the mean uterine weights of the animals injected with two and three pituitaries do not differ significantly. The injection of acetone-dried pitui-

XUM

taries of 20 day old cockerels did not increase the uterine weights although the dosage varied considerably. Since previous work had indicated this to be one of the critical periods in pituitary-gonad relationship, these data were likewise a disappointment. The acetone-dried weight of the pituitary at the 20th day is about one-sixth of the fresh weight and, therefore, the largest dosage in these series was equivalent to approximately 6 mg. of fresh gland (about 3 pituitaries). If it is necessary to administer this much or more glandular substance to produce significant increases the test is limited in its value for fowl assay. A study of the vaginal epithelium—using stage 2 as indicative of stimulation as suggested by Riley and Fraaps (1942)—showed the same lack of quantitative differentiation and poor responsiveness to pituitaries of younger birds.

SUMMARY

Assays of gonadotrophic content of cockerel anterior pituitaries were made using the 96-hour chick test. Excellent results were achieved and a "chick unit" C.U. was established which represented a 35% increase in gonad weight of the injected animals over the control where 10 or more experimental animals were used. No significant differences were noted between acetone-dried pituitaries and fresh glands when these were injected on the basis of equivalent weight of fresh gland.

Gonadotropic potency was determined for entire glands and per milligram weight of the glands. Total potency ranged from 0.5 C.U. on the fifth day to 8.3 C.U. on the 90th day and the per milligram potency from 0.19 C.U. on day 23 to 1.0 C.U. on day 90. Considerable fluctuation in the per milligram potency of the glands was noted and these variations were compared with the fluctuations in testes weights. The testes weights expressed as a percentage of body weight increased approximately 700% during the period studied. There was a correlation between the per milligram potency curve and that for the testes weights. When there was a plateau in the testes curve the per milligram potency of the glands increased but when the testes weights increased the curve of milligram potency either decreased or plateaued. These data demonstrate that there is an early secretion by both the anterior pituitary and the testes in the White Leghorn cockerel and that an intimate interrelationship exists between the secretory activity of these glands.

A comparative test of pituitary potency was made on mice using the uterine weight increase for assay. Good responses were elicited but the quantitative responses were not satisfactory. Little difference was noted in several instances between series which received 1, 2 and 3 pituitaries and likewise slight differences were observed between pituitaries of several age periods.

REFERENCES

BRENEMAN, W. R.: *Endocrinology* 26: 1091. 1940.
BRENEMAN, W. R.: *Endocrinology* 35: 456. 1944.
BYERLY, T. C., AND W. H. BURROWS: *Endocrinology* 22: 366. 1938.
CLARK, H. M.: *Anat. Rec.* 61: 175. 1935.
JAAP, R. GEORGE: *Poult. Sci.* 14: 237. 1935.
KUPPERMAN, H. S., W. H. ELDER AND R. K. MEYER: *Endocrinology* 29: 23. 1941.
MEYER, C., H. MELLISH AND H. S. KUPPERMAN: *J. Pharmacol. & Exper. Therap.* 65: 104. 1939.
PAYNE, FERNANDUS: *Biol. Bull.* 82: 79. 1942.
PHILLIPS, R. E.: *Poult. Sci.* 21: 161. 1942.
RIDDLE, OSCAR: *Endocrinology* 15: 307. 1931.
RIDDLE, OSCAR, AND J. P. SCHOOLEY: *Proc. Soc. Exper. Biol. & Med.* 32: 1610. 1935.
RILEY, G. M., AND R. M. FRAAPS: *Endocrinology* 30: 529. 1942.

XUM

THE RELATIVE THYROIDAL POTENCY OF l- AND d,l-THYROXINE[1]

E. P. REINEKE AND C. W. TURNER
From the Department of Dairy Husbandry, University of Missouri
COLUMBIA, MISSOURI

ALTHOUGH it was established by Harington and Salter (1930) that thyroxine naturally occurs in the thyroid in the optically active levo form, there is still considerable uncertainty as to the relative potency of pure l-thyroxine and the racemic thyroxine that until recently has been the only form readily available for animal experimentation and clinical use.

Using compounds prepared by Harington (1928) Gaddum reported that, as tested by the stimulation of tadpole metamorphosis (1927) and rat metabolism (1929–30), l-thyroxine showed about three times the potency of the d- form. Upon testing the same compounds in human myxedema, Salter, Lerman and Means (1935) found no difference in activity. On the other hand Foster, Palmer and Leland (1936) reported that l-thyroxine obtained by the enzymatic hydrolysis of thyroid substance exerted twice the effect of a racemic mixture as judged by its calorigenic effect in guinea pigs, and thus inferred that the d- compound is devoid of activity. Likewise Reineke and Turner (1943b) reported that l-thyroxine isolated from iodinated casein exerted twice the metabolic effect of a racemic mixture when tested on guinea pigs.

With both l- and d,l-thyroxine now easily obtainable by isolation from thyroidally active iodinated casein as reported by Reineke and Turner (1943a, 1943b), it seemed desirable to extend these observations to additional species in order to determine whether or not the differences noted above could be ascribed to differences in species response.

MATERIALS AND METHODS

The crystalline l-thyroxine used was taken from a lot isolated from iodinated casein subsequent to hydrolysis with sulfuric acid as reported previously (Reineke and Turner, 1943b). It showed a specific rotation $[\alpha]_D = -4.2°$, a melting point of 236–238° C., and contained 65.1% iodine. Crystalline d,l-thyroxine was obtained by isolation from iodinated casein after hydrolysis with barium hydroxide (Reineke and Turner, 1943a). It

Received for publication November 17, 1944.
[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 952.

was highly purified, and was found by spectrographic analysis to be identical with pure synthetic thyroxine.

Stock solutions of l- and d,l-thyroxine were prepared by dissolving 10 mg. in a 100 ml. volumetric flask with the aid of N/10 sodium hydroxide and then adding just sufficient N/10 hydrochloric acid to cause precipitation of the thyroxine as the monosodium salt. These solutions were stored at 5° C., and were replaced with freshly prepared solutions at least once a week. Dilute solutions were prepared immediately before injection by pipetting well-mixed aliquots of the stock solution, dissolving with a drop of N/10 sodium hydroxide and making up to the desired volume. Any solution remaining from day to day was discarded.

The chick trials were conducted with male white Plymouth Rock chicks placed on experiment when about 48 hours old, and given 0.1 per cent thiouracil in their feed as described by Mixner, Reineke and Turner (1944). By this technique the thiouracil inhibits the formation of hormone by the chick's own thyroid, permitting an increased output of thyrotrophic hormone by the pituitary and compensatory hyperplasia of the thyroid gland. If thyroxine is injected simultaneously with the thiouracil treatment the thyrotrophic hormone is held in check, and thyroid enlargement is prevented in greater or lesser degree in proportion to the amount of thyroxine injected. Since we have also observed that the thyroid response of chicks can be affected markedly by seasonal factors (unpublished) the data to be reported were all obtained within a period of about a month (Nov. 1943) when no seasonal changes were noted.

A second comparison was made on albino rats of the Missouri strain and ranging from about 150 to 300 gms. in body weight. Thiouracil was given as 0.1% of the drinking water, and graded doses of l- and d,l-thyroxine were injected subcutaneously daily during a 14-day period in order to determine their relative potency in reducing the size of the thyroid, according to the technique first reported by Dempsey and Astwood (1943). Each animal was weighed daily before injection and the thyroxine dosage was adjusted in proportion to body weight. It has been shown by Reineke, Mixner and Turner (1944) that the decrease in thyroid weight with increasing dosage of thyroxine under these conditions compares closely with the increase in metabolic rate.

The thyroidal activity of the two forms of thyroxine was also compared on frog tadpoles (Rana Pipiens) as described by Reineke and Turner (1942). Large tadpoles approximately 60 to 70 mm. in length were collected for this purpose. Fresh thyroxine solutions were prepared immediately before injection. Each tadpole was measured with a millimeter rule, given a single injection of thyroxine in a volume of 0.1 ml. of solution and placed in an individual container. They were measured again 3 days later, after visible metamorphosis had occurred, and the response was estimated by the mean percentage decrease in body length of the various groups.

RESULTS

In the three species used as test animals, l-thyroxine uniformly showed approximately twice the potency of the racemic mixture.

When the average chick thyroid weights (log scale) are plotted against the daily thyroxine dosage (arithmetic scale) such that 1 unit

of l-thyroxine is equal to 2 units of racemic thyroxine the two response curves are superimposable (Fig. 1). Thus at any dosage level l-thyroxine exhibits twice the thyroidal activity of the same amount of the racemic compound.

The average rat thyroid weights (Fig. 2) are plotted by the same

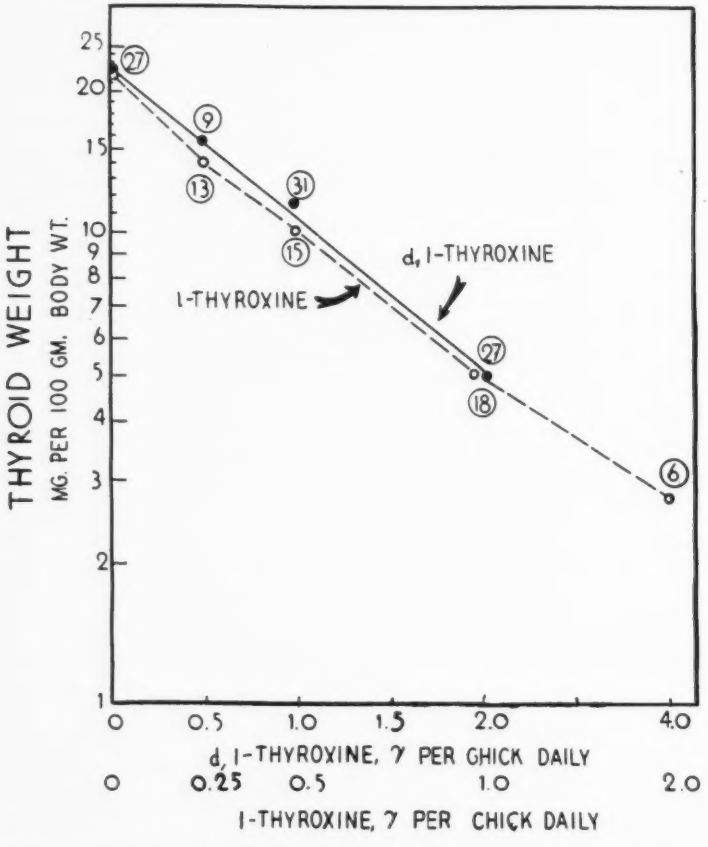


FIG. 1. The relative potency of l- and d,l-thyroxine in reducing the thyroid weight of thiouracil-treated male chicks. The encircled numerals in this and the other charts indicate the number of animals per dosage group.

method. Data for both male and female rats were grouped together, since separate treatment showed no significant differences between the response of the two sexes. Here again the thyroxine response curves follow the same course, although somewhat less uniformly than was observed with the chick data. The thyroid weight reduction on 1 and 2 micrograms, respectively, of d,l-thyroxine is slightly greater

than that on 0.5 and 1.0 micrograms of l-thyroxine. However, 1.5 micrograms of l-thyroxine produced a slightly greater response than 3 micrograms of d,l-mixture. Thus, within the limits of experimental variation, l-thyroxine shows twice the potency of the racemic form

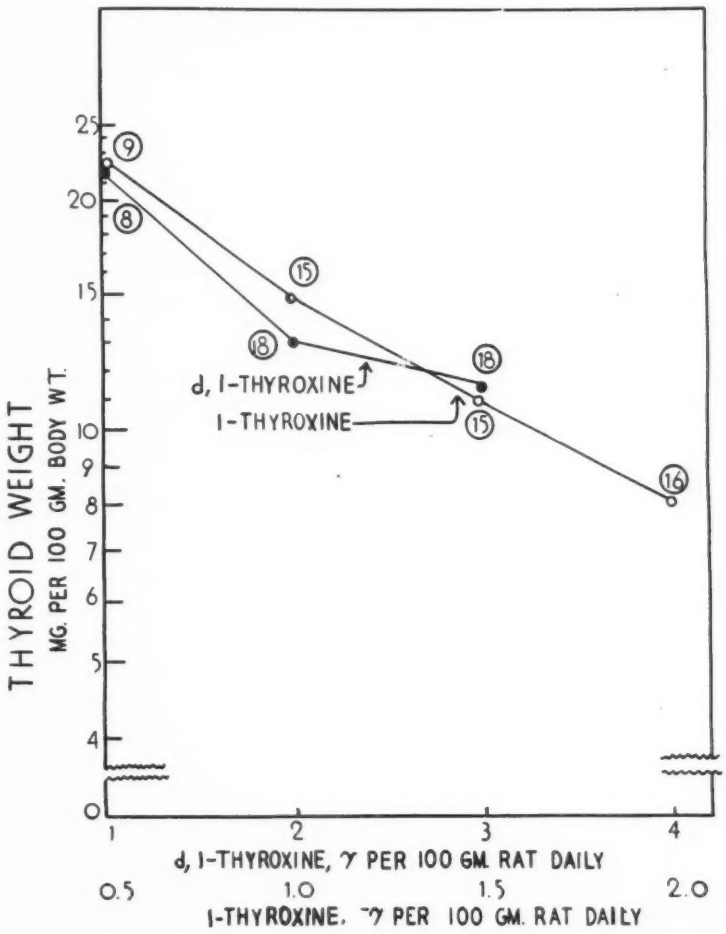


FIG. 2. The relative potency of l- and d,l-thyroxine in reducing the thyroid weight of thiouracil-treated rats.

when tested by its thyroid weight reducing power in thiouracil-treated rats.

This is confirmed further by the results obtained when the two thyroxine preparations were compared for their potency in stimulating the metamorphosis of frog tadpoles (Fig. 3). The average percent-

age decrease in body length of groups of tadpoles is plotted against log dose, and the dosage scale is adjusted again so that 1 unit of l-thyroxine is equivalent to 2 units of racemic mixture. Throughout the range of dosage a given amount of l-thyroxine produces an effect equal to that elicited by twice the amount of d,l-thyroxine.

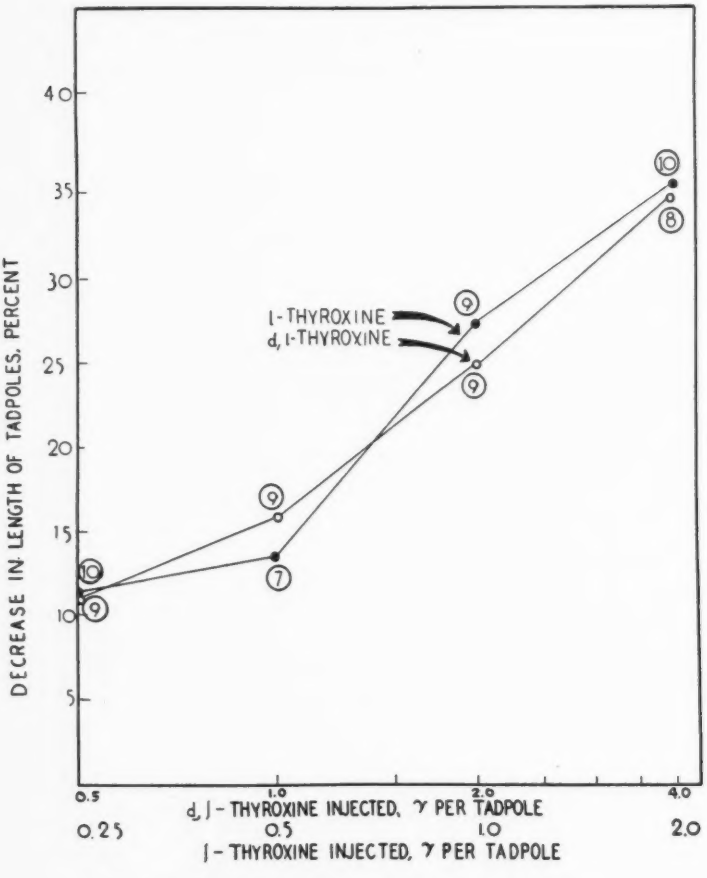


FIG. 3. The relative potency of l- and d,l-thyroxine in stimulating the metamorphosis of tadpoles.

DISCUSSION

As judged by comparative assays on chicks, rats and tadpoles, l-thyroxine was found to exert twice the thyroidal effect of the d,l-mixture. This confirms and extends the previous reports of Foster, Palmer and Leland (1936) and Reineke and Turner (1943b) who

found that l-thyroxine produced twice the calorigenic effect of the racemic compound when tested on guinea pigs.

It is difficult to reconcile these results with those of Gaddum who reported that the d- form had $\frac{1}{3}$ of the potency of l-thyroxine when tested on tadpoles (1927) and rats (1929–30), two of the same species employed in the present investigations. According to Gaddum's results l-thyroxine should exert only 1.5 times the effect of the racemic mixture whereas our trials show the activity ratio of l- to d,l-thyroxine to be 2 to 1. Assuming this to be the true relationship, since it was confirmed in separate trials on four different species of test animals, it can be inferred that all or substantially all of the activity of d,l-thyroxine resides in the l-compound, and that d-thyroxine has little or no activity. Even though we had no pure d-thyroxine available for direct testing, the difference between our results and those of Gaddum are too great to be accounted for in the experimental variation of the assay methods used. Looking elsewhere for an explanation, we come to the question of the purity of the compounds tested. Our l-thyroxine as well as that isolated by Leland, Foster and Palmer showed a higher specific rotation, using a sodium vapor light source, than that prepared by Harington (1928) and tested by Gaddum. As pointed out previously by Leland, *et al.*, the specific rotation of the latter compound was determined with a mercury green light which ordinarily yields higher values than sodium vapor light. This strongly suggests that the resolution of the d- and l-thyroxine was somewhat less than complete, which could account for the activity reported for the d-compound. In fact in his original report Harington recognized the possibility that the biological activity shown by his d-thyroxine may have been due to contamination with the l- form.

No evidence for a species difference in the response to l- and d,l-thyroxine was found in tests with 4 different types of laboratory animals. From the report of Salter, Lerman and Means (1935) it appears that man is unique in failing to differentiate between the two forms of thyroxine. In view of the foregoing, however, it would seem desirable that this question be investigated more fully before final conclusions are drawn.

As discussed in detail by Foster, Palmer and Leland (1936) the discrepancy that has been repeatedly observed between the thyroxine content of thyroid tissue and its biological activity when expressed in terms of a d,l-thyroxine standard is eliminated if it is recognized that the form occurring in the thyroid has the l- configuration and consequently exhibits twice the activity of the d,l-form ordinarily available as a standard.

It is also important that this concept be kept in mind in evaluating biological measurements of thyroid hormone output by the technique of Dempsey and Astwood (1943) or Mixner, Reineke and Turner (1944). Although no physical constants that would establish

XUM

its identity are reported, Dempsey and Astwood state that they used l-thyroxine as a standard for their measurements of thyroid hormone in rats, and they find a thyroid hormone output equivalent to approximately 5 micrograms of l-thyroxine. Reineke, Mixner and Turner (1944) determined the thyroid hormone output of male rats of the same weight and kept at approximately the same environmental conditions, and found a thyroid secretion equivalent to 4.8 micrograms of d,l-thyroxine. Thus in view of the 2:1 potency ratio of l- and d,l-thyroxine, this, instead of being in close agreement, would represent a difference of nearly 100 per cent in the estimated thyroid hormone production of the rats used in the two laboratories. Insufficient data are at hand to determine the reason for this difference. It would be of interest, however, to have a more complete characterization of the thyroxine used by the former workers to determine whether it was, in fact, the l- compound.

SUMMARY AND CONCLUSIONS

The comparative thyroidal activity of l- and d,l-thyroxine were determined by their relative ability to reduce the thyroid weight of thiouracil-treated chicks and rats and by their stimulation of metamorphosis in frog tadpoles. Confirming results reported previously on guinea pigs, it was found that l-thyroxine produced twice the effect of the racemic mixture in every species tested. From this it is concluded that all of the activity of racemic thyroxine can be accounted for by its l- component and that the d- compound must have little or no activity.

REFERENCES

Dempsey, E. W., and E. B. Astwood: *Endocrinology* 32: 509. 1943.
Foster, G. L., W. W. Palmer and J. P. Leland: *J. Biol Chem.* 115: 467. 1936.
Gaddum, J. H.: *J. Physiol.* 64: 246. 1927.
Gaddum, J. H.: *J. Physiol.* 68: 383 1929–30.
Harington, C. R.: *Biochem. J.* 22: 1429. 1928.
Harington, C. R., and W. T. Salter: *Biochem. J.* 24: 456. 1930.
Mixner, J. P., E. P. Reineke and C. W. Turner: *Endocrinology* 34: 168. 1944.
Reineke, E. P., and C. W. Turner: *Mo. Agr. Exp. Sta. Bul.* 355. 1942.
Reineke, E. P., and C. W. Turner: *J. Biol. Chem.* 149: 555. 1943a.
Reineke, E. P., and C. W. Turner: *J. Biol. Chem.* 149: 563. 1943b.
Reineke, E. P., J. P. Mixner and C. W. Turner. *Endocrinology* 36: 64. 1945.
Salter, W. T., J. Lerman and J. H. Means: *J. Clin. Investigation* 14: 37. 1935.

THE PITUITARY ADRENOTROPHIC HORMONE CONTROL OF THE RATE OF RELEASE OF SERUM GLOBULINS FROM LYMPHOID TISSUE[1]

ABRAHAM WHITE AND THOMAS F. DOUGHERTY
From the Departments of Physiological Chemistry and Anatomy, Yale University
NEW HAVEN, CONNECTICUT

THE SYNTHESIS in the liver of the albumin of the blood has been well established, whereas a significant portion of serum globulins may be produced extra-hepatically. A generalized production of the blood globulins in the cells of the reticulo-endothelial system has been suggested by numerous investigators, and has been chiefly indicated from studies of antibody production and from clinical data.

Recently, interest in the physiology of plasma proteins has been manifested particularly with respect to factors influencing their regeneration. Emphasis has been on the parenteral utilization of various sources of protein nitrogen. The proteins of tissues have also received attention as a possible source of plasma protein, and Whipple and his colleagues (Madden and Whipple, 1940; Whipple, 1938, 1942; Whipple and Madden, 1944) have suggested the existence of a dynamic equilibrium between blood and tissue proteins.

The hypothesis has recently been suggested that the rate of lymphoid tissue dissolution is normally under pituitary-adrenal cortical control (White and Dougherty, 1944). Acceleration of this process by pituitary adrenotrophic hormone, or by adrenal cortical extracts, is reflected in a contribution of a portion of the protoplasmic nitrogen of the cellular constituents of lymphoid tissue to the blood in the form of protein (White and Dougherty, 1944). This added protein has been assumed to belong, in part, to the portions of the blood proteins containing antibodies, since the rate of antibody release from the lymphoid tissue of immunized rabbits was demonstrated to be under pituitary-adrenal cortex control (Dougherty, Chase and White, 1944a). The time correlations existing, after adrenotrophic hormone or adrenal cortical extract injections, among histological changes in lymphoid tissue (White and Dougherty, 1944),

Received for publication December 26, 1944.

[1] Presented in part before The American Chemical Society, New York, September, 1944.

This investigation was aided by grants from the Josiah Macy, Jr. Foundation and from the Fluid Research Fund, Yale University School of Medicine.

blood lymphopenia (Dougherty and White, 1944) increases in total serum protein (White and Dougherty, 1944) and augmented antibody titers (Dougherty, Chase and White, 1944a) strongly suggest that pituitary-adrenal cortical activity is the normal mechanism controlling the rate of release of at least a portion of the serum globulin from reticulo-endothelial cells in general and, specifically, from lymphoid tissue. This tissue, therefore, represents an important site of synthesis of certain of the blood globulins and may, indeed, be part of the reserve or deposit protein which investigators have long discussed. A portion of this protein released in augmented amounts as a result of any one of the variety of pituitary-adrenal cortical stimulants, might function as antibody in an immunized animal, as protein for gluconeogenesis, as a source of amino acids (in the liver) for the synthesis of albumin, and as protein contributing to the maintenance of circulating plasma volume through its effective colloidal osmotic pressure.

The experiments described in this paper establish the increases in total proteins of the blood of mice and rats following injection of pituitary adrenotrophic hormone, the lowering of the total serum proteins of mice following adrenalectomy, and the increase in the beta and gamma globulin fractions of the blood proteins produced in rabbits by single injections of either adrenotrophic hormone or adrenal cortical steroids in oil. Further, protein extracts from isolated rabbit lymphocytes contain a protein with an electrophoretic mobility, at pH 7.95 and ionic strength 0.2, identical with the mobility of the gamma globulin of normal rabbit serum under the same conditions. The data establish that lymphoid tissue is an important site of formation of one or more of the blood globulins, and demonstrate the pituitary-adrenal cortical control of the release of these proteins from lymphoid structures.

MATERIALS AND METHODS

Adult mice (CBA strain, Strong) of both sexes were used in these studies. The rats were males of the Sprague-Dawley strain and weighed approximately 200 to 250 gm. when used. The rabbits were males of mixed parentage and weighed approximately 2.5 kg. Food and water were available to the animals at all times during the studies, the mice and rats being fed Purina Fox Chow and the rabbits the same food supplemented with oats.

Tail vein blood of mice and rats and heart blood of rabbits were used for analysis.[2] The specific gravity of whole blood was determined by the falling drop method of Kagan (1941). Total serum protein values were also obtained by the falling drop technique (Kagan, 1938) and checked by the Kjeldahl method for total nitrogen, using a protein factor of 6.25. An arbitrary correction factor of 30 mg. of non-protein nitrogen per 100 ml. of blood was deducted from the total nitrogen. Satisfactory agreement between the

[2] Tail vein blood was used in mice for determining whole blood specific gravity. When this blood had been obtained, each mouse was decapitated with a sharp scissors and the free flowing blood collected for serum protein estimations.

data was obtained by the two methods of estimating total serum proteins.

The Tiselius apparatus employed is equipped with the Philpot scanning device (Philpot, 1938). All electrophoresis experiments have been conducted under the following conditions: bath temperature, 3° C., buffer, 0.02 molar phosphate containing 0.1 molar sodium chloride, pH 7.95, and a potential-gradient of 5 to 6 volts cm.$^{-1}$ The rabbit sera were diluted with 2 volumes of the buffer and dialyzed for 48 hr. at 3° C. against 2 liters of the same buffer. Each electrophoresis was permitted to continue until complete separation of the protein components had occurred. The magnified projections of the diagrams obtained were traced and the areas under the peaks measured by means of a planimeter. Areas are expressed in relation to one another in terms of per cent. Absolute amounts of each fraction in grams could then be calculated from the total protein contents of the sera.

Protein-containing extracts of lymphocytes were prepared in the following manner. Thymus glands, mesenteric lymph nodes, and popliteal nodes of rabbits were carefully freed of extraneous tissue and finely minced with sharp scissors. Pooled tissue from several rabbits was employed for each experiment. The minced material was diluted with several volumes of physiological saline and centrifuged. The cellular sediment was washed three additional times in a similar manner. The final supernatant was completely free of cells. An imprint of the sediment was made and microscopically showed intact, well-formed cells. A differential cell count, using a smear of the sediment stained with May-Grünwald Giemsa, showed that 90 per cent of the cells were lymphocytes. These cells were then lysed by mixing with one volume of distilled water and grinding the mixture in a mortar, with the addition of a small amount of sand. One volume of 20 per cent sodium chloride was added and the grinding and extraction continued vigorously for approximately one hour. Good separation of solvent and residue could not be obtained by ordinary centrifugation, due to the thick, viscous nature of the mixture. However, ultracentrifugation at 15,000 r.p.m. for 30 minutes provided adequate quantities of clear supernatant for further study. These extracts were dialyzed against several changes of the phosphate buffer used in the electrophoresis studies and then examined in the Tiselius apparatus.

Extracts obtained in this manner were slightly red in color and exhibited a high degree of viscosity. Their approximate protein content (nitrogen $\times 6.25$) varied from 1.5 to 3 per cent. The use of larger volumes of saline for extraction yielded more dilute and less viscous extracts, but the protein content was too low for satisfactory Tiselius studies. This could be circumvented by dialyzing the more dilute extracts against distilled water until free of salt, and drying the mixture in the dialysis bag by the lyophile process. The powders obtained in this manner yielded the same Tiselius patterns as those obtained with extracts dialyzed against buffer and examined electrophoretically without preliminary concentration. It may be pointed out that approximately 30 per cent of such extracts, and of the lyophilized powders, were insoluble in the particular buffer solution which has been employed.

The adrenotrophic hormone employed in these studies was a highly purified preparation from hog pituitaries (Sayers, White and Long, 1943). A potent concentrate of adrenal cortical steroids in oil was kindly supplied by Dr. E. Gifford Upjohn of the Upjohn Company. The desoxycorticosterone acetate in sesame oil is a product of the Schering Corporation.

RESULTS

Injections of adrenotrophic hormone in rats and mice. Table 1 contains data showing the changes in total serum proteins occurring in the rat after a single subcutaneous injection of 3.0 mg. of adrenotrophic hormone, and in the mouse after 15 days of daily, subcutaneous injections of 1.0 mg. of the hormone. Within 3 hours after a single dose of hormone, the rat responds with a significant increase in total serum proteins which is still evident 6 hours following the injection. At 24 hours after hormone administration the total serum protein value has returned to approximately normal. No significant changes in whole blood specific gravity occurred in these animals and the specific gravity data are, therefore, not included in the table.

TABLE 1. EFFECT OF PITUITARY ADRENOTROPHIC HORMONE AND OF ADRENALECTOMY ON TOTAL SERUM PROTEINS

Animal	No. of animals	Treatment	Total Serum proteins gm/%	P values[1]
Normal rat	22	———	6.00 ± 0.01[2]	
Normal rat	8	3 hrs. after 3 mg. A[3]	6.22 ± 0.07	<0.01
Normal rat	8	6 hrs. after 3 mg. A	6.30 ± 0.08	<0.01
Normal rat	4	24 hrs. after 3 mg. A	5.81 ± 0.01	0.04
Normal mouse	19	———	6.02 ± 0.02	
Normal mouse	17	1 mg. A daily for 15 days	6.52 ± 0.07	<0.01
Adrenalectomized mouse	16	0.025 mg. D[4] daily, 8 days	5.59 ± 0.08	<0.01

[1] P values, compared to controls.
[2] Means and standard errors
[3] A = adrenotrophic hormone.
[4] D = desoxycorticosterone acetate.

Daily hormone injections in the mice produced a consistently elevated serum protein level.

The alterations in serum proteins may not appear to be of great magnitude, although statistically highly significant. However, when these serum protein increases are considered in the light of the circulation time of the blood, and the period over which they are maintained, it is apparent that protein is being added to the blood at an increased rate as a consequence of stimulation of the adrenal cortex by the pituitary adrenotrophic hormone.

Effect of Adrenalectomy. Table 1 also contains data demonstrating that absence of the adrenals, and the consequent lack of the normal hormones concerned with controlling the rate of release of serum protein from lymphoid tissue (White and Dougherty, 1944), results in a hypoproteinemia under the conditions described. The daily administration of desoxycorticosterone acetate, a steroid which does not produce a lymphopenia (Dougherty and White, 1944) or lymphoid tissue involution in the doses used (White and Dougherty, 1944) prevented marked hemoconcentration in the adrenalectomized mouse.

In the absence of desoxycorticosterone administration, hemoconcentration may obscure the serum protein fall.

Injection of adrenotrophic hormone or adrenal cortical steroids in rabbits.—Normal rabbits were given single, subcutaneous injections of either 10 mg. of adrenotrophic hormone (in 2 ml. water) or 5 ml. of a solution of adrenal cortical steroids in oil. Blood was obtained from the rabbits at 3, 6, 9, 12, and 24 hours after hormone injection, only one blood sample being taken from each rabbit. Each serum was subjected to electrophoresis in the Tiselius apparatus. The data obtained from each serum were very similar, regardless of the time interval after hormone injection which the serum represented. Conse-

TABLE 2. SERUM PROTEIN CHANGES IN RABBITS FOLLOWING SUBCUTANEOUS INJECTION OF ADRENOTROPHIC HORMONE OR ADRENAL CORTICAL STEROIDS

	Controls	Adrenotrophic hormone injected (10 mg.)	P values[1]	Adrenal cortical steroids injected in oil (5 ml.)	P values[1]
No. of Animals	5	7		5	
Total protein	5.50 ± 0.24[2]	6.13 ± 0.18[2]	0.05	5.62 ± 0.37[2]	0.8
% albumin	72.9 ± 9.2	62.3 ± 2.6	0.02	61.1 ± 1.4	<0.01
% globulin	27.1 ± 9.2	37.7 ± 2.6	0.02	38.9 ± 1.4	<0.01
% α globulin	4.22 ± 0.30	3.94 ± 0.60	0.75	4.77 ± 0.46	0.5
% β globulin	12.43 ± 1.6	16.52 ± 2.2	0.05	15.80 ± 1.5	0.15
% γ globulin	10.42 ± 1.15	17.28 ± 1.5	<0.01	18.32 ± 1.12	<0.01
gm. albumin	4.03 ± 0.30	3.81 ± 0.13	0.5	3.44 ± 0.25	0.15
gm. globulin	1.47 ± 0.12	2.32 ± 0.20	<0.01	2.18 ± 0.13	<0.01
gm. α globulin	0.23 ± 0.03	0.23 ± 0.04	>0.9	0.27 ± 0.03	0.30
gm. β globulin	0.67 ± 0.08	1.03 ± 0.18	<0.01	0.88 ± 0.03	0.09
gm. γ globulin	0.56 ± 0.04	1.07 ± 0.02	<0.01	1.03 ± 0.03	<0.01

[1] P values as compared to controls.
[2] Values in these columns are means and standard errors.

quently, in table 2 all the rabbit data are grouped together without regard to the time after injection of hormone that the blood was drawn.

The percentage compositions of the sera in Table 2 were calculated from the electrophoretic patterns. From these values, the absolute number of grams of each protein fraction could be obtained, knowing the total protein content. The absolute values clearly show that a single injection of either pituitary adrenotrophic hormone or adrenal cortical steroids in oil in normal rabbits produces, during the 24 hour period following hormone administration, a significant increase in total serum globulin. This increase occurs in both the beta and gamma globulin fractions and is most striking in the latter. The gamma globulin fraction normally carries a large portion of blood antibodies. These data conform with the demonstration (Dougherty, Chase and White, 1944a) that the rate of release of immune substances from lymphoid tissue is under pituitary-adrenal cortex control. Apparently in the

XUM

normal, non-immunized rabbit, this rate of release of globulin is also under similar control and is accelerated when excess hormone is injected.

Electrophoresis of lymphoid cell extracts.—Electrophoresis studies have been conducted with extracts prepared from the lymphoid cells of normal rabbits and from rabbits injected with adrenotrophic hor-

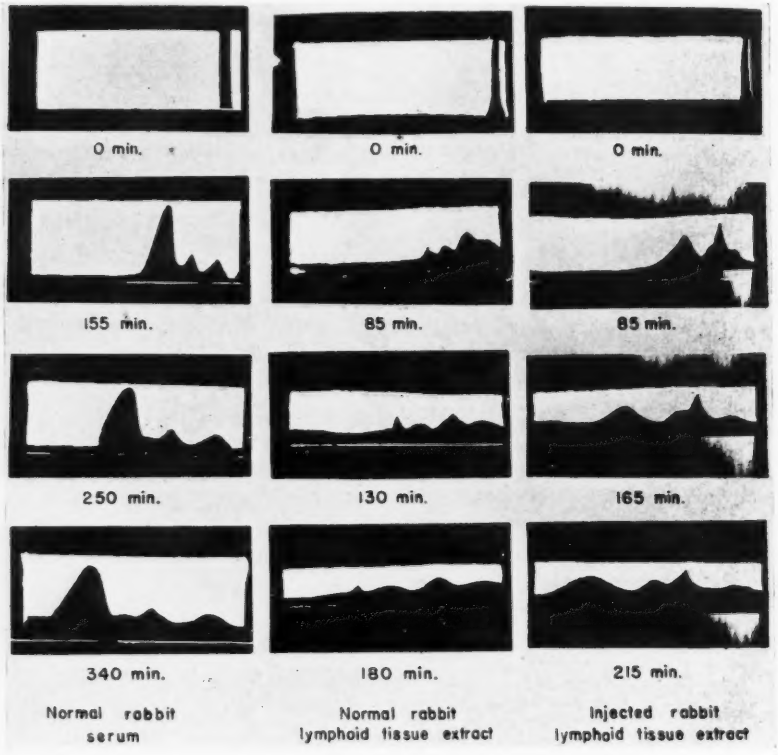


FIG. 1. Electrophoretic patterns of normal rabbit serum and of lymphoid tissue extracts. All photographs of descending limb at times indicated. Temp. 3° C., pH 7.95; 0.02 molar phosphate buffer containing 0.1 molar sodium chloride. Potential-gradient 5 to 6 volts cm.$^{-1}$

mone or with a solution of adrenal cortical steroids in oil. The electrophoretic patterns for the cell extracts obtained from uninjected as compared to injected rabbits resemble one another in the number and in the mobilities of the protein components present. However, in the extracts of lymphoid cells from the injected rabbits there is a striking increase in the quantity of one of the proteins, that having the highest mobility. These electrophoretic patterns are illustrated

in figure 1, which also includes an electrophoretic pattern of normal rabbit serum for purposes of comparison.

Under the conditions of the Tiselius experiments, the slowest moving component of lymphoid cell extracts has an electrophoretic mobility identical with that of the slowest moving protein fraction of normal rabbit serum, the gamma globulin. The comparison of these mobilities is given in table 3. These data, together with the evidence of the pituitary adrenal cortex control of antibody release from lymphoid tissue (Dougherty, Chase and White, 1944a), its correlation with the lymphoid tissue dissolution (White and Dougherty, 1944), and the demonstration of labelled globulin, in the form of antibody, in the lymphocytes of immunized mice (Dougherty, Chase and White, 1944b), clearly establish the lymphocytes of the lymphoid tissue as a normal source of the gamma globulin of the blood serum. The rate of

TABLE 3. ELECTROPHORETIC MOBILITY OF SLOWEST MOVING PROTEIN COMPONENT IN NORMAL RABBIT SERUM AND IN LYMPHOID CELL EXTRACTS

Material	No. of determinations	Mobility (μ) $\times 10^5$ cm.^{2} volt^{-1} sec.$^{-1}$	P value[1]
Normal rabbit serum	2	0.67 ± 0.10[2]	
Lymphoid cell extracts	4	0.71 ± 0.02	>0.9

[1] P value as compared to normal rabbit serum.
[2] Means and standard errors.

release of the protein from lymphocytes is under pituitary adrenotrophic hormone control and is normally effected by the liberation of the protein of the lymphocyte as a consequence of increased adrenal cortical steroid secretion.

DISCUSSION

The increase in total serum proteins occurring in the rat following adrenotrophic hormone injection was apparent at three and six hours after hormone administration. Twenty-four hours after the injection, the serum protein level was approximately normal. In the rabbits, hormone injection produced a rise in serum globulins which persisted throughout the three to twenty-four hour periods that the animals' sera were studied in the Tiselius apparatus. Beyond this time, total serum protein values frequently were lower than those seen in normal rabbits. The quantity of total circulating protein might have been actually at an elevated level for periods beyond the twenty-four hour interval following hormone injection. This may not have been evident in determinations of total serum proteins since these have now been diluted as a result of the plasma volume increase (Dougherty and White, 1944).

The data presented are acute changes occurring at definite time intervals following hormone injection, particularly before a twenty-

XUM

four hour period had elapsed. In studies of continued hormone administration, additional factors, e.g., the nutritional status of the experimental subject, and therefore the capacity to replace protein, and simultaneous metabolic demands on the body, may also greatly influence the type of blood protein response seen after hormone. In clinical cases additional circumstances may intervene. Unpublished results have shown similar increases in total serum proteins in hospital patients three hours after injection of adrenal cortical steroids in oil.[3] This increase is not evident twenty-four hours after the hormone has been given.

The data which have been obtained establish the pituitary adrenal cortical control of the rate of release of protein from lymphoid tissue and possibly other tissues. It has been previously demonstrated (Dougherty, Chase and White, 1944a) that this mechanism regulates the rate of production and release of certain antibodies. Furthermore, the present study shows that both the beta and the gamma globulin fractions of the blood are increased in normal rabbits in the twenty-four hour period following injections of either adrenotrophic hormone or adrenal cortical steroids. That the lymphocytes can be a source of a portion of this globulin is seen from the fact that lymphocytes contain a protein which is electrophoretically similar to the normal serum gamma globulin. The recent demonstration of the presence of antibodies in lymphocytes (Dougherty, Chase and White, 1944b) supports the electrophoretic data. This body of evidence, correlated in time relationships with the alterations in lymphoid tissue histology occurring after hormone injection (White and Dougherty, 1944; unpublished results) provides the basis for the conclusion that lymphoid tissue is a normal physiological reservoir of a portion of the blood globulins whose rate of release to the circulation is under pituitary adrenal cortical control. In a real sense, therefore, lymphoid tissue and other aggregates of lymphoid cells may represent a portion of the reserve or deposit body protein which has long been discussed. Cannon, Humphreys, Wissler and Frazier (1944) have recently shown that bovine serum gamma globulin is highly efficient with respect to serum protein regeneration in the hypoproteinemic rat. Lymphoid tissues, therefore, assume added metabolic importance since they may provide gamma globulin, a protein of a high nutritional quality. It is possible that the observed effectiveness of gamma globulin in restoring serum protein levels may be related to its contribution to depleted protein reserves which, in part, have an amino acid composition identical with the serum gamma globulin.

The hypothesis that lymphoid tissue is a source of at least a portion of the blood globulin is in harmony with the idea of an extra hepatic source of globulin. Albumin formation, on the other hand,

[3] These studies were conducted in cooperation with Dr. E. A. Lawrence and Barbara W. Patton.

occurs chiefly in the liver. Thus, liver damage in experimental or clinical circumstances results in a hypoalbuminemia with frequently no alteration in the level of blood globulin, or, indeed, an increase in globulin concentration (Berryman and Bollman, 1943; Cardon and Atlas, 1943; Erickson, Heckel and Knutti, 1938; Gray and Barron, 1943; Kagan, 1943). Globulin increases under these circumstances may result from an augmented rate of protein release from lymphoid tissue as a result of the stimulation of pituitary adrenal cortical activity by the toxic agents responsible for the liver damage or accumulating because of liver insufficiency.

Adrenalectomy in the mouse was followed by a decrease in total serum proteins which may be overlooked if hemoconcentration is not prevented. Food intake of operated animals and the length of the postoperative period preceding serum protein analysis are also variables which must be adequately considered in studies of the effect of adrenalectomy or of hypophysectomy on the serum protein picture. It should be emphasized that the pituitary adrenotrophic hormone is the normal regulator of the rate of the processes which have been discussed and that hormone injection has, therefore, increased this rate. In the absence of the adrenals or the pituitary, lymphoid tissue hyperplasia occurs (Dougherty and White, unpublished results; Reinhardt and Holmes, 1940) because the mechanism regulating the normal rate of lymphoid tissue dissolution is absent and therefore lymphoid tissue attains a maximum size. However, this dissolution does not entirely cease (Dougherty and White, unpublished results). Consequently, the mass of lymphoid tissue and potential serum protein is greatly increased in the operated animal. In this event an actual increase of serum globulin above normal might eventually obtain, despite the absence of the pituitary or the adrenals.

A portion of the globulin being continually released from lymphoid tissue may be converted in the liver to carbohydrate. This gives added meaning to the established role of the adrenal cortex in gluconeogenesis. It is also conceivable that some of this metabolically available nitrogen may serve as source material for serum albumin synthesis. Therefore, hypophysectomy or adrenalectomy may be followed also by changes in serum albumin concentration which are secondary to the prime controlling influence of these endocrine glands on lymphoid tissue.

Experimental and clinical literature contain numerous examples of serum protein changes which may now be interpreted on the basis of the preceding discussion. A few examples may be cited of hyperglobulinemia concomittant with increased pituitary adrenal cortical function. Certain infections affect the adrenal cortex (Sarason, 1943; Sayers, Sayers, Fry, White and Long, 1944) and are also accompanied by hyperglobulinemia (Bing, 1940; Cardon and Atlas, 1943; Kagan, 1943; Longsworth, Shedlovsky and MacInnes, 1939). This is, of course,

XUM

also seen in a striking manner during immunization with infectious material. Fasting will augment adrenal cortical secretion (Sayers, Sayers, Fry, White and Long, 1944). In this connection it is interesting that rabbits fasted for twenty-four hours had total blood globulin and gamma globulin concentrations distinctly higher (Sharp, Taylor, Beard and Beard, 1942) than those found by Seibert and Nelson (1942, 1943), whose data for normal fed rabbits are in agreement with the data for control rabbits in this paper. Trauma produces marked adrenal cortical activity (Sayers, Sayers, Fry, White and Long, 1944), and Chanutin, Hortenstine, Cole and Ludewig (1938) observed an immediate and sustained rise in the plasma globulin of the rat following a control laparotomy. These authors concluded that "the operative procedure of laparotomy alone stimulates globulin production at once and for a long period." Elliott (1914) reported the depletion of the stainable lipid of the adrenal of the human following severe hemorrhage; similar observations were made in the rat by Engel, Winton and Long (1943). A significant quantity of the protein restored to the circulation following hemorrhage is contributed from the lymphatics (Cotui, Barcham and Shafiroff, 1944; Shafiroff, Doubilet, Preiss and Cotui, 1943). Other conditions causing mechanical or chemical stress may result in augmented adrenal activity and would conceivably cause similar alterations in the blood protein pattern. Many of these stimuli have been studied by other investigators.

The data which have been presented have established that lymphocytes are a normal source of at least a portion of the blood globulin and that the rate of release of this protein is under the physiological control of the pituitary adrenotrophic hormone. In an immunized animal some of the lymphocyte protein is antibody (Dougherty, Chase and White, 1944b). The findings emphasize a mode of the functioning of the lymphocyte in its hitherto unexplained role in resistance to infectious diseases, transplantation, and other conditions of stress. Thus, the role of the adrenal cortex in these circumstances is related to its controlling influence over lymphoid tissue.

SUMMARY

Single injections of pituitary adrenotrophic hormone in rats resulted in an increase in total serum proteins measured at three or six hours after injection. Twenty-four hours after hormone administration, the total serum proteins had returned to approximately normal levels. Continued daily injection of adrenotrophic hormone for fifteen days in the mouse produced a consistently elevated serum protein level. Adrenalectomy in mice gave evidence of significantly lowered total protein content of the sera of the operated animals, provided marked hemoconcentration was prevented.

Single injections of adrenotrophic hormone or of adrenal cortical steroids in rabbits produced, during the three to twenty-four hour

period following hormone administration, a significantly increased concentration of total serum globulin, due to significant increases in the beta and gamma globulin fractions of the bloods of the injected rabbits.

Electrophoretic examination of the protein-containing extracts of washed, lysed lymphocytes from rabbit lymphoid tissue revealed the presence of a protein component with an electrophoretic mobility identical with that of the gamma globulin of normal rabbit serum at the same pH (7.95) and ionic strength (0.2).

The data lead to the conclusion that the lymphocytes of lymphoid tissue are a storehouse for a portion of the globulin fraction of the serum, and that the rate of release of this protein is under the normal physiological control of the pituitary adrenotrophic hormone which exerts its influence by way of the adrenal cortex. The evidence permits the integration of the functioning of the lymphocyte and the adrenal cortex in normal defense mechanisms of the organism.

REFERENCES

Berryman, J. H., and J. L. Bollman: *Am. J. Physiol.* 139: 592. 1943.

Bing, J.: *Acta med Scandinav.* 31: 3143. 1940.

Cannon, P. R., E. M. Humphreys, R. W. Wissler and L. E. Frazier: *J. Clin. Investigation* 23: 601. 1944.

Cardon, L., and D. H. Atlas: *Arch. Int. Med.* 71: 377. 1943.

Chanutin, A., J. C. Hortenstine, W. S. Cole and S. Ludewig: *J. Biol. Chem.* 123: 247. 1938.

Cotui, I. S. Barcham and B. G. P. Shafiroff: *Surg., Gynec. & Obst.* 79: 37. 1944.

Dougherty, T. F., J. H. Chase and A. White: *Proc. Soc. Exper. Biol. & Med.* 56: 28. 1944a.

Dougherty, T. F., J. H. Chase and A. White: *Proc. Soc. Exper. Biol. & Med.* 57: 295. 1944b.

Dougherty, T. F., and A. White: *Endocrinology* 35: 1. 1944.

Dougherty, T. F., and A. White: unpublished results.

Elliott, T. R.: *Quart. J. Med.* 8: 47. 1914.

Engel, F. L., M. G. Winton and C. N. H. Long: *J. Exper. Med.* 77: 397. 1943.

Erickson, C. C., G. P. Heckel and R. E. Knutti: *Am. J. Path.* 14: 537. 1938.

Gray, S. J., and E. S. G. Barron: *J. Clin. Investigation* 22: 191. 1943.

Kagan, B. M.: *J. Clin. Investigation* 17: 373. 1938.

Kagan, B. M.: *J. Lab. & Clin. Med.* 26: 1681. 1941.

Kagan, B. M.: *Am. J. M. Sc.* 206: 309. 1943.

Longsworth, L. G., T. Shedlovsky and D. MacInnes: *J. Exper. Med.* 70: 399. 1939.

Madden, S. C., and G. H. Whipple: *Physiol. Rev.* 20: 194. 1940.

Philpot, J. S. L.: *Nature*, London 141: 283. 1938.

Reinhardt, W. O., and R. O. Holmes: *Proc. Soc. Exper. Biol. & Med.* 45: 267. 1940.

Sarason, E. L.: *Arch. Int. Med.* 71: 702. 1943.

Sayers, G., M. A. Sayers, E. G. Fry, A. White and C. N. H. Long: *Yale J. Biol. & Med.* 16: 361. 1944.

Sayers, G., A. White and C. N. H. Long: *J. Biol. Chem.* 149: 425. 1943.

Seibert, F. B., and J. W. Nelson: *J. Biol. Chem.* 143: 29. 1942.

Seibert, F. B., and J. W. Nelson: *Am. Rev. Tuberc.* 47: 66. 1943.

Shafiroff, B. G., H. Doubilet, A. L. Preiss and Cotui: *Surg., Gynec. & Obst.* 76: 547. 1943.

Sharp, D. G., A. R. Taylor, D. W. Beard and J. W. Beard: *J. Immunol.* 44: 115. 1942.

Whipple, G. H.: *Am. J. M. Sc.* 196: 609. 1938.

Whipple, G. H.: *Am. J. M. Sc.* 203: 477. 1942.

Whipple, G. H., and S. C. Madden: *Med.* 23: 215. 1944.

White, A., and T. F. Dougherty: *Proc. Soc. Exper. Biol. & Med.* 56: 26. 1944.

XUM

THE RELATIVE POTENCY OF SOME ADRENAL CORTICAL STEROIDS IN THE MUSCLE-WORK TEST

DWIGHT J. INGLE AND M. H. KUIZENGA
From the Research Laboratories, The Upjohn Company
KALAMAZOO, MICHIGAN

THE PURPOSE of this paper is three-fold. First, data are reported which show that under the conditions of a muscle-work test (Ingle, 1944) the compounds 17-hydroxycorticosterone, 17-hydroxy-11-dehydrocorticosterone, corticosterone, 11-dehydrocorticosterone and 11-desoxycorticosterone acetate can be ranked from most potent to least potent in the order named. Second, consideration is given to the data and conclusions of Vögtli (1943a, 1943b) which have challenged the validity of the muscle-work test as a measure of cortical hormone activity. Third, some of the experimental findings relative to the differences in biologic properties of the C-11 oxygenated cortical steroids and 11-desoxycorticosterone are briefly reviewed.

METHODS

Male rats of the Sprague-Dawley strain which weighed 180 ± 2 gm. were adrenalectomized and nephrectomized in a single stage operation and immediately subjected to the work test. The apparatus and procedures were identical with those previously described (Ingle, 1944). Subcutaneous injections of the hormones were made at the beginning of stimulation and again six hours later. Stimulation was continued either until the muscle ceased to respond or for a period of twenty-four hours. The average total number of recorder revolutions for the twenty-four period was used in the calculation of "units" from a previously established standard curve (Ingle, 1944).

EXPERIMENTS AND RESULTS

In Experiment I (Figure I, Table I), the activities of the four C-11-oyxgenated cortical steroids were tested in parallel. When the activity equivalents of work performance were read from the standard dose-response curve (Ingle, 1944) the following values were obtained: At the level of 0.2 mgm. per dose 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone assayed 7.3 units and 5.8 units per mgm., respectively, while at the level of 0.5 mgm. per dose corticosterone and 11-dehydrocorticosterone assayed 2.7 and 1.9 units per mgm. The value of 5.8 units per mgm. for 17-hydroxy-11-dehydro-

Received for publication December 29, 1944.

corticosterone is higher than the value of 5.0 units per mgm. which was assigned by the definition of a unit (Ingle, 1944). Such a difference falls within the expected limits of error of this assay method. Since the comparisons of these four compounds were made under optimal

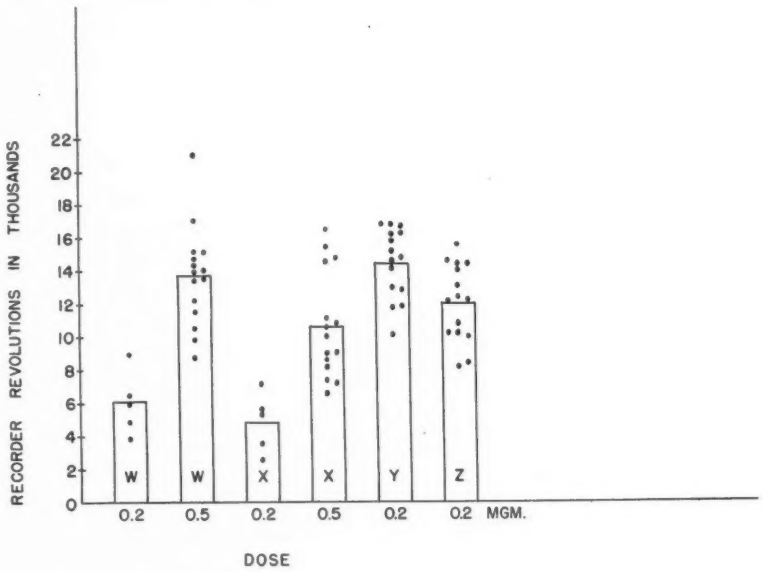


FIG. 1. *Work Performance as Related to the Dosage of the C-11-oxygenated Cortical Steroids.* Each recorder revolution is equal to approximately 400 gram centimeters of work.

W corticosterone
X 11-dehydrocorticosterone
Y 17-hydroxycorticosterone
Z 17-hydroxy-11-dehydrocorticosterone

conditions, the values for activity which were calculated from the standard dose-response curve were "corrected" in order to correspond to the value of 5.0 units per mgm. which was assigned to 17-hydroxy-

TABLE I. AVERAGES AND VARIABILITY OF THE VALUES FOR TOTAL WORK

Compound	Dose mg.	N rats	Units per mgm.	Mean value for work	Sigma distribution	Sigma mean	Diff. between means	Sigma diff.	Diff. sigma diff.
Corticosterone	0.5	15	2.3	13667	2902	751			
11-dehydrocorticosterone	0.5	15	1.6	10658	3085	795	3009	1092	2.75
17-hydroxycorticosterone	0.2	15	6.3	14454	2069	534			
17-hydroxy-11-dehydro-corticosterone	0.2	15	5.0	12060	2298	593	2394	798	3.00

Work is expressed as number of recorder revolutions. Each recorder revolution is the equivalent of approximately 400 gram-centimeters work.

The ratio of 2.75 between the difference and the sigma of the difference means, on a statistical basis, that chances are 997 in 1000 that the true difference is greater than zero. The ratio of 3.0 means that the chances are 999 in 1000 that the true difference is greater than zero.

11-dehydrocorticosterone by definition. The ratio between the assigned value, 5.0 units per mgm. and the assay value of 5.8 units per mgm. is 1.0 to 1.16. All assay values were divided by 1.16 to give the following corrected values: 17-hydroxycorticosterone, 6.3 units per mgm.; 17-hydroxy-11-dehydrocorticosterone, 5.0 units per mgm.; corticosterone, 2.3 units per mgm., and 11-dehydrocorticosterone, 1.6

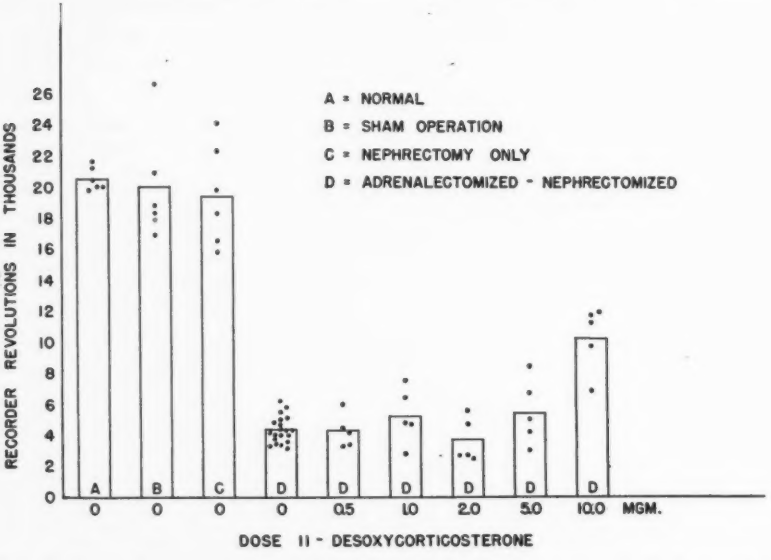


FIG. 2. *Group Averages and Individual Records of Work Performance.* Each recorder revolution is equal to approximately 400 gram-centimeters of work.

units per mgm. The analysis of the data (Table I) was made in terms of work performance and indicates that the direction of the differences between any two values is probably reliable.

Experiment 2, (Figure 2) was a comparison of the work performance of normal, sham-operated, nephrectomized and adrenalectomized-nephrectomized rats. In non-adrenalectomized animals the sham-operation and excision of the kidneys caused only small and possibly insignificant decreases in work performance. The performance of untreated adrenalectomized-nephrectomized animals was very poor and 11-desoxycorticosterone acetate had little or no effect on work in all but large doses.

The values for work by the normal rats were also recorded at the end of eight hours of stimulation. The average initial rate of work was twenty-four recorder revolutions per minute and at the end of eight hours it had decreased to eighteen recorder revolutions per minute. The average total work at the end of eight hours was 9717 recorder

revolutions or the equivalent of 21.59 kilogram-meters per 100 grams of rat.

DISCUSSION

In this work performance test significant differences in quantitative effects have been shown among the C-11-oxygenated cortical steroids. All of those studied were much more effective than 11-desoxycorticosterone acetate. However, the biological effect of a compound is a consequence of the nature of both the test substance and the test object and may be influenced by all of the experimental conditions. There are many examples of the same compounds showing different ratios of activity when compared by different methods. It would be unsound to generalize about the relative quantitative effects of these steroids from the results of either this or any one assay method.

The data from a number of other studies support the conclusion that the C-11-oxygenated cortical steroids differ in their quantitative effects. Ingle (1940a) studied the effects of these same compounds on the work performance of adrenalectomized rats prior to the standardization of the work test as an assay method. There was no differentiation between 11-dehydrocorticosterone and corticosterone but 17-hydroxy-11-dehydrocorticosterone gave a greater response than either of the two first named compounds and 17-hydroxycorticosterone gave the greatest response of all. Since the numbers of animals involved were small and the compounds were not tested in parallel no claim was made as to the reliability of the observed differences. The results of Grattan and Jensen (1940) indicate that the glycogenic effect of 17-hydroxycorticosterone was greater than that of corticosterone in the fasting mouse. Swingle *et al.* (1942) observed in a single adrenalectomized dog that the protection against circulatory failure afforded by corticosterone seemed distinctly inferior to that of 17-hydroxy-11-dehydrocorticosterone. Reinecke and Kendall (1943) compared corticosterone, 11-dehydrocorticosterone and 17-hydroxy-11-dehydrocorticosterone by means of glycogenic test in fasting adrenalectomized rats. It was concluded that there was probably less than a onefold difference among their respective potencies. The actual differences, although small in magnitude and lacking statistical significance, were of the same relative order found in the present study. Olson, Thayer and Kopp (1944) used a sensitive and carefully standardized glycogenic test in adrenalectomized, fasting rats. No significant difference was found between the potencies of corticosterone and 11-dehydrocorticosterone, 17-hydroxy-11-dehydrocorticosterone induced a greater response of questionable significance, and 17-hydroxycorticosterone, the most potent of all, had a significantly greater glycogenic effect than corticosterone. Finally, Ingle, Winter, Sheppard and Kuizenga (to be published) have compared corticosterone and 17-hydroxycorticosterone for diabetogenic action and effect on

nitrogen balance in force-fed normal rats. As judged by these criteria, 17-hydroxycorticosterone was the more potent of the two compounds.

From the present study and these related studies it may be fair to make the tentative generalization that the addition of a hydroxyl group in the C-17 position enhances the effect of the C-11-oxygenated compounds on the metabolism of carbohydrate and related functions which are involved in maintaining the ability of adrenalectomized rats to work. Although the results of the present study indicate that the potency of such compounds is greater when the oxygen at C-11 is represented by a hydroxyl instead of a keto group, such a conclusion is less well supported by the data of other studies.

The data of this study support the earlier conclusions of Ingle (1940a) that under these experimental conditions 11-desoxycorticosterone is much less effective than the C-11-oxygenated compounds but that large doses of 11-desoxycorticosterone do produce a positive effect on work.

Vögtli (1943a, 1943b) has studied the muscle work test of Ingle and reached the conclusion that, contrary to the results of Ingle (1940a, 1940b, 1940c), 11-desoxycorticosterone is not qualitatively deficient in its ability to sustain work output in adrenalectomized rats. The criticisms and conclusions of Vögtli are unacceptable to the present writers. The principal considerations relative to these studies are summarized as follows:

First, Vögtli used the discharge of a condenser as a stimulus to muscle instead of the stimulator described by Heron, Hales and Ingle (1934) and used thereafter by Ingle until its recent modification (Ingle, 1944). The fact that the type of stimulation used was a highly important factor in determining the work of muscle and that this apparatus was selected only after a number of less effective devices were tested was pointed out by Heron, Hales and Ingle (1934). No method was less effective in our hands than stimulation by the discharge of a condensor. The duration of the discharge is too brief. Although strong contractions could be induced at the beginning of stimulation it was impossible to sustain an optimal rate of work. A comparison of the work performance of normal rats over an eight hour period in the experiments of Vögtli with the work performance of normal rats of similar weight in the present experiments is significant. Vögtli reported an average value of 5.57 kilogram-meters per 100 grams or rat whereas the average value obtained in the present study was 21.59 kilogram-meters per 100 gram of rat. Inspection of the work curves of Vögtli show that at the end of the eight hour period of stimulation the height of muscular contraction was approximately 25 per cent of the initial height of contraction. In the present study the average height of muscular contraction at the end of eight hours of stimulation was 75 per cent of the initial height of contraction. One of us (Ingle) has reviewed the values for work attained by normal

and sham-operated animals in earlier experiments and found that all values were far higher than those reported by Vögtli.

Second, Vögtli stimulated the muscles of his test animals for only eight hours whereas we have used a minimum period of twenty-four hours. It was stated by Vögtli that the period of stimulation was limited to eight hours because "unavoidable" infections and continued narcosis cannot serve for physiological comparisons. The type of infections encountered by Vögtli were not described. In our experiments we have consistently kept intact and operated animals working continuously at a high rate for several days without infections or any untoward effects of continued narcosis. The homogeneity of performance has been as good as in most physiological studies and the test animals have remained sensitive to changes in experimental conditions. Very early in our work we encountered considerable difficulty from respiratory infections. Sniffles, a common infection in laboratory rats, frequently caused plugging of the nose and trachea with mucus so that asphyxia resulted. This problem was solved prior to the first report of our work. The success of our procedures requires the careful selection of healthy test animals. More recently we have been able to maintain a colony of rats completely free from all infections to use in these and other studies. As shown by Hales, Haselrud and Ingle (1935), the stimulation of muscle for eight hours does not suffice to demonstrate a wide difference between the performance of animals having the adrenal glands intact and those from which the adrenals have been removed. Similarly, in the experiments of Vögtli the difference in work performance between normal and adrenalectomized animals was very small as compared to the several-fold differences which we have consistently obtained in our studies. In later studies we have intensified the stress to which the animals are subjected so that no untreated adrenalectomized animal is able to work as long as twenty-four hours.

Third, Vögtli has stated that the early decrease in work output of adrenalectomized animals immediately following operation was due to operative shock rather than adrenal cortical insufficiency. In his experiments, sham-operated and adrenalectomized rats showed the same small decrease below normal by eight hours. The chemical data (Vögtli, 1943b) support the contention that his sham-operated animals developed shock. Vögtli failed to give recognition to any of the control data which we have published (Hales, Haselrud and Ingle, 1935; Ingle, 1935; Ingle, 1940a and b; Ingle and Lukens, 1941). In all of our experiments the performance of sham-operated rats and of rats subjected to other severe control operations has been as high or almost as high as that of unoperated animals.

Fourth, it was claimed by Vögtli that the treatment of adrenalectomized rats with 11-desoxycorticosterone acetate for several days prior to the work test was effective in restoring a normal work per-

XUM

formance thereby confirming the hypothesis that the ineffectiveness of this steroid in the acute tests was due to slow absorption. Vögtli omitted consideration of the discussions of the same hypothesis by Ingle or of the experiments which were made to test the hypothesis. Ingle (1940b) compared the work performance of adrenalectomized rats treated for one week with 11-desoxycorticosterone and its acetate with the effects of 17-hydroxy-11-dehydrocorticosterone and its acetate. Although the rats treated with 11-desoxycorticosterone and its acetate performed a great deal more work than untreated animals, especially during the early hours of stimulation, the total amounts of work were very small as compared to the effects of 17-hydroxy-11-dehydrocorticosterone and its acetate. These large differences were fully revealed by prolonged stimulation by an optimal form of stimulation. The results which Vögtli obtained by employing a suboptimal stimulus for as short a period as eight hours might have been anticipated from our own experiments. Another study is relative to this problem. Ingle (1941c) studied the effect of the highly water-soluble 11-desoxycorticosterone phosphate on work performance and found it to be ineffective under conditions in which 17-hydroxy-11-dehydrocorticosterone has a striking effect.

Clinical and laboratory investigators have not been fully agreed as to the biological activities of 11-desoxycorticosterone. Verzar (1941) has contended that the apparent deficiencies of 11-desoxycorticosterone as compared to the C-11-oxygenated compounds are due to its slower rate of absorption and that during longer periods of administration 11-desoxycorticosterone will fully replace the functional activity of the adrenal cortices. These conclusions are opposed by the results of many careful investigations from other laboratories and clinics. On the other hand, there are many statements in the literature that this compound is completely devoid of any effect upon carbohydrate metabolism. It is our opinion that both of these opposing concepts are based upon misconceptions and that 11-desoxycorticosterone influences the carbohydrate metabolism of adrenalectomized animals in at least two different ways. First, treatment of adrenal cortical insufficiency by either 11-desoxycorticosterone or by an optimal intake of electrolytes tends to restore normality to the circulatory mechanisms which are essential for the support of all phases of carbohydrate metabolism. Anderson (1943) has reviewed the favorable effects of high sodium chloride intake upon adrenalectomized animals. Second, 11-desoxycorticosterone is weakly active in stimulating the formation of carbohydrate from non-carbohydrate sources and possibly in modifying the utilization of carbohydrate. Large doses of this steroid have a positive effect upon work performance as shown in this study. Ingle and Thorn (1940) demonstrated that large doses exerted a diabetogenic effect in partially depancreatized rats. Harrison

and Harrison (1939) have observed that large doses of the compound were capable of sustaining a normal level of blood glucose in fasting, adrenalectomized rats. These effects of 11-desoxycorticosterone are more likely to be manifest in chronic rather than acute experiments and the factor of absorption may be important in experiments with this steroid.

Although treatment of adrenal cortical insufficiency by means of 11-desoxycorticosterone may either greatly improve or fully restore to normal the blood and tissue stores of carbohydrate under certain experimental conditions where the physiological demands for carbohydrate are minimal the latent inadequacy of this compound as compared to the C-11-oxygenated compounds is revealed in any situation in which the demands for carbohydrate are unusually high and prolonged. Thus, the diabetic animal (Ingle and Thorn, 1940) having its glycosuria decreased or abolished by adrenalectomy does not show a restoration of the preoperative level of glycosuria during treatment with the usual therapeutic doses of 11-desoxycorticosterone. The normal ability of muscle to respond to severe stimulation for many hours is not sustained by treatment with the usual life-maintenance doses of 11-desoxycorticosterone. As concluded by Swingle *et al.* (1942) certain types of experimental shock are apparently based upon some defect in carbohydrate metabolism and are not significantly benefited by 11-desoxycorticosterone acetate. Thorn (1942) and MacBryde and de la Balze (1944) are among the many clinical investigators who have reported that some patients having Addison's disease are prone to develop hypoglycemia during fasting and are unresponsive to the usual treatment with 11-desoxycorticosterone. In all of these situations the C-11-oxygenated cortical steroids are effective in restoring carbohydrate metabolism to normal.

SUMMARY

The biologically active adrenal cortical steroids were compared as to their effect upon the ability of adrenalectomized rats to work. Under the conditions of this study, 17-hydroxycorticosterone was the most active compound, 17-hydroxy-11-dehydrocorticosterone was second, corticosterone was third, 11-dehydrocorticosterone was fourth and 11-desoxycorticosterone was much less active than any of the C-11-oxygenated compounds. It was shown by new data and by review of earlier studies that the decreased ability of adrenalectomized rats to work is due to adrenal cortical insufficiency and not to operative shock.

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. E. Schwenk of the Schering Corporation, Bloomfield, New Jersey, who supplied the 11-desoxycorticosterone acetate.

REFERENCES

ANDERSON, E.: Essays in Biology. pp. 35. University of California Press. Berkeley & Los Angeles 1943.
GRATTAN, J. F., AND H. JENSEN: *J. Biol. Chem.* 135: 511. 1940.
HALES, W. M., G. M. HASELRUD AND D. J. INGLE: *Am. J. Physiol.* 112: 65. 1935.
HARRISON, H. E., AND H. C. HARRISON: *Proc. Soc. Exper. Biol. & Med.* 42: 506. 1939.
HERON, W. T., W. M. HALES AND D. J. INGLE: *Am. J. Physiol.* 110: 357. 1934.
INGLE, D. J.: *Am. J. Physiol.* 116: 622. 1936.
INGLE, D. J.: *Endocrinology* 26: 472. 1940a.
INGLE, D. J.: *Endocrinology* 27: 297. 1940b.
INGLE, D. J.: *Proc. Soc. Exper. Biol. & Med.* 44: 450. 1940c.
INGLE, D. J.: *Endocrinology* 34: 191. 1944.
INGLE, D. J., AND F. D. W. LUKENS: *Endocrinology* 29: 443. 1941.
INGLE, D. J., AND G. W. THORN: *Am. J. Physiol.* 132: 670. 1941.
INGLE, D. J., H. A. WINTER, R. SHEPPARD AND M. H. KUIZENGA: (To be published).
MACBRYDE, C. M., AND F. A. DE LA BALZE: *J. Clin. Endocrinology* 4: 287. 1944.
OLSON, R. E., S. A. THAYER AND L. J. KOPP: *Endocrinology* 35: 464. 1944.
REINECKE, R. M., AND E. C. KENDALL: *Endocrinology* 32: 505. 1943.
SWINGLE, W. W., J. W. REMINGTON, V. A. DRILL AND W. KLEINBERG: *Am. J. Physiol.* 136: 567. 1942.
THORN, G. W.: *J. Mt. Sinai Hosp.* 8: 1177. 1942.
VERZAR, F.: *Schweiz. med. Wchnschr.* 71: 358. 1941.
VÖGTLI, W.: *Helv. Physiol. Acta* 1: 393. 1943a.
VÖGTLI, W.: *Helv. Physiol. Acta.* 1: 407. 1943b.

EFFECTS OF YEAST OR WATER SOLUBLE VITAMINS IN EXPERIMENTAL PANCREATIC DIABETES

O. H. GAEBLER AND WALTER E. CISZEWSKI
From the Department of Laboratories, Henry Ford Hospital
DETROIT, MICHIGAN

DIETARY FACTORS AND GLYCOSURIA

SINCE YEAST CELLS metabolize glucose very actively, it has long been supposed that yeast might contain substances of value in the treatment of diabetes. Beneficial effects from oral administration of yeast to diabetics were reported even before the present century, but they may simply have indicated that a patient with diabetes and vitamin deficiency shows some improvement if the latter condition alone is treated. Lusk (1931) commented that the enzymes of yeast could not be absorbed.

From various sources including yeast, Collip (1923) prepared extracts which, when given subcutaneously, lowered the blood sugar of normal rabbits and depancreatized dogs, and also controlled glycosuria in the latter test animal. He postulated the existence in plants of an insulin-like hormone, which he called glucokinin. Other investigators found similarities between the effects of insulin and B-complex upon protein metabolism, and even chemical identity of insulin and "vitamin B" was seriously considered. The literature on this point is reviewed by Nasset, Pierce, and Murlin (1931), whose experiments demonstrated the favorable effect of yeast upon nitrogen storage, both in normal and in depancreatized dogs, and indicated a slight decrease of the D:N ratio in some of the latter animals.

Experiments on intact rats (Mattill, 1923) gave no indication that water soluble vitamins were involved in carbohydrate metabolism. Burack and Cowgill (1932) found carbohydrate tolerance to be diminished in intact dogs deprived of water soluble vitamins, but presented good evidence that this was due to inanition.

Meanwhile direct studies have linked a number of water soluble vitamins with enzyme systems involved in oxidation and fermentation of glucose. Reviewing this development, Dienst (1939) stated that the beneficial action of yeast in diabetes had been known since 1898, and reported noteworthy experiments on 6 diabetics. His patients received diets of known carbohydrate, fat, and protein content. After a control

Received for publication January 4, 1945.

period of several weeks, the diets were supplemented with a preparation containing thiamine, ascorbic acid, and the entire B group. Carbohydrate tolerance improved in all cases; the daily insulin requirement fell as much as 20 U., and fluctuations in blood sugar level were reduced. Dienst considered simultaneous addition of the entire B group merely a condition which permitted the added thiamine to have its optimal effect. He ascribed his striking results to thiamine and ascorbic acid.

Martin (1937) found that the insulin requirement of depancreatized dogs increased continuously during development of extreme multiple vitamin deficiency, and that choline alone did not improve matters. He fed diets containing much carbohydrate. Eventually 60 U. of insulin daily in 1 animal, and 160 U. daily in 2 others, left the glycosuria entirely out of control. After the first animal's weight had fallen from 12.3 kg. to 7.5 kg., dramatic improvement followed simultaneous administration of all the vitamins, and moderate doses of insulin were again effective. The other 2 animals were lost. Experiments with individual vitamins were included, but are difficult to interpret. In connection with the action of yeast, alimentary absorption of glucokinin was among the possibilities that Martin considered.

In the following experiments we observed the effect of withdrawing yeast from the diet of depancreatized dogs receiving a constant amount of food and insulin, and tried to reproduce the beneficial effect of yeast with known vitamins. Lipocaic deficiency and deficiency of fat soluble vitamins were excluded. Fasting diabetes was kept out of the picture so far as possible by terminating any deficiency which led to refusal of part of the food. Insofar as development of glycosuria corresponds to reduction of food intake the element of inanition may have appeared, but in any event inanition was not a factor in the origin of the glycosuria. The requirement that food intake be constant of course determined the degree of B group deficiency that could be studied. The diet was somewhat unusual in that it contained as much fat and carbohydrate as Cowgill's diet (Cowgill, 1923) for normal dogs.

EXPERIMENTAL

The following basal diet was used in all experiments, with one exception to be mentioned later, in which vitamin-free casein replaced commercial casein.

Commercial casein	15 lbs. or 6800 gm.
Cracker meal	15 lbs. or 6800 gm.
Mazola (corn oil)	4000 ml.
Calcium phosphate, dibasic, powdered	900 gm.
Salt mixture (Cowgill, 1923)	228 gm.
Haliver oil, 15 drops daily	
Pancreatin, 15 gm. daily	

The ingredients other than haliver oil and pancreatin were mixed mechanically, and the oily powder was stored in closed containers. The average

analysis indicated 36.7 per cent of protein, 23.1 per cent of ether extract, and 29.1 per cent of starch. One animal received 180 gm., and 2 others 200 gm., of the mixture daily. This amounted to 55 to 65 calories per kg. There were 2 meals daily, each moistened with 200 ml. of tap water, which the animals also received *ad. lib.*

The dose of haliver oil proved to be more than adequate, for concentrations of 200 μg. of vitamin A per 100 ml. appeared in the blood. To simplify feeding, we prepared separate lots of food identical with the above diet, but containing in addition 750 gm. of dried brewers' yeast, strain C, from Anheuser Busch Brewing Company. This addition increased the quantity of mixture without notably altering the composition. Since animals received the same weight of each diet, cessation or resumption of yeast feeding made no change of consequence in caloric intake. Thiamine hydrochloride, riboflavin, nicotinic acid, and inositol were purchased from Eastman Kodak Company, and pyridoxin from Merck and Company. Sodium pantothenate was purchased from Parke Davis and Company, in 10 mg. capsules, and ascorbic acid from Hoffman LaRoche, in 25 mg. tablets. The specified amount of pancreatin caused very complete digestion and absorption. The stools were firm, and could be collected quantitatively, yet the nitrogen content was usually below 1 gm. per day.

All pancreatectomies appeared to be complete at operation, and under our dietary conditions omission of even a single dose of insulin resulted in severe glycosuria. Since all the animals are still being used, no autopsies are available. The insulin requirement of each animal was determined in long preliminary observations. Both protamine and plain insulin, injected separately, were used to control glycosuria with a minimal total dose. The analytical methods were the same as in the preceding study (Gaebler and Tarnowski, 1943).

RESULTS

Glycosuria, Nitrogen Balance, and Weight

Tables 1, 2, and 3 represent continuous observations on 1 animal for 283 days. The daily averages are given for periods which, with very few exceptions, lasted 3 days. Data on the other 2 animals will be discussed in the section on blood sugar changes, ketosis, and lipemia.

In the experiment shown in Table 1, progressive glycosuria began during the fifth period following withdrawal of yeast, and eventually reached a high plateau. It was accompanied by loss of weight, marked rise of urine nitrogen, and negative nitrogen balances. The glycosuria did not at once disappear when yeast feeding was resumed, but did so gradually in 12 days. A second withdrawal and replacement of yeast produced the same sequence of events.

Attempts to replace yeast with pure vitamins are shown in Table 2. Yeast was withdrawn on January 29, and replaced by amounts of thiamine, riboflavin, and nicotinic acid equal to the guaranteed content of the yeast. After a temporary decline, glucose output increased almost as much as in the preceding experiment in which nothing was given in place of yeast. The animal also lost nitrogen, and was brought

into balance again by feeding yeast. On March 2 yeast was replaced by 4 times the amounts of thiamine, riboflavin, and nicotinic acid used in the preceding experiment. The animal did not lose weight, and no rise in urine nitrogen occurred for 27 days, after which occasional small negative balances were found. Glycosuria developed later

TABLE 1. EFFECTS OF YEAST IN A DEPANCREATIZED BITCH RECEIVING A CONSTANT AMOUNT OF FOOD AND INSULIN

Date	Weight	Nitrogen intake	Urine nitrogen	Nitrogen balance	Urine sugar	Diet and remarks
1943–4	kg.	gm. per day	gm. per day	gm. per day	gm. per day	
10/26–28	14.0	10.87	9.01	1.24	0	7 gm. yeast daily since July,
10/29–31		10.87	8.44	1.91	2.4	yeast discontinued 11/1
11/ 1–3		10.92	9.45	0.64	1.1	
11/ 4–6	14.1	10.92	9.01	1.44	1.3	
11/ 7–9	14.2	10.92	9.36	0.74	2.2	
11/10–12	14.2	10.92	9.86	0.31	5.0	
11/13–15	14.2	10.92	8.99	1.10	13.7	
11/16–18	14.1	10.92	11.33	−1.34	36.3	
11/19–21	13.9	10.98	11.57	−1.15	50.3	
11/22–24	13.7	11.01	10.07	−0.02	60.7	
11/25–27	13.7	10.81	10.71	−0.67	70.4	7 gm. yeast daily
11/28–30	13.8	11.85	10.69	−0.13	52.5	22 gm. yeast daily
12/ 1–3	14.0	11.85	10.67	0.11	25.1	22 gm. yeast daily
12/ 4–6	14.3	11.85	10.26	0.08	7.7	22 gm. yeast daily
12/ 8–10	14.3	10.81	9.99	−0.05	0	7 gm. yeast daily
12/11–13	14.3	11.01	8.90	1.30	0.6	Yeast discontinued
12/14–16	14.4	11.01	9.30	0.62	1.5	
12/17–19	14.4	11.01	8.19	1.93	2.6	
12/20–22	14.2	11.01	10.06	−0.01	8.6	
12/23–25	14.2	11.01	8.96		15.3	
12/26–29	14.2	10.87	11.22	−1.18	44.7	
12/30–1/1	14.0	10.81	10.55	−0.26	68.9	7 gm. yeast daily from 12/30
1/ 2–4	14.1	10.85	10.29	−0.54	48.9	to 1/28 (Table 2)
1/ 5–7	14.1	10.88	10.10	−0.03	47.1	
1/ 8–10	14.2	10.88	9.10	0.88	22.3	
1/11–13	14.3	10.88	9.69	0.09	15.5	
1/14–16	14.3	10.88	9.66	0.20	9.3	
1/17–19	14.5	10.88	9.82	−0.05	6.6	
1/20–22	14.4	10.87	8.73	1.22	11.8	

This animal received daily 195 gm. of the basal diet, including pancreatin and haliver oil. After the morning meal 8 U. of protamine insulin and 3 U. of plain insulin were given separately. After the evening meal, 3 U. of plain insulin were given. Stool nitrogen was determined throughout the experiment but only the balances are recorded.

than before, but became almost as serious, and was not arrested by addition of sodium pantothenate and ascorbic acid to the diet.

Accordingly feeding of p-aminobenzoic acid, pyridoxin, and inositol was begun on April 30 (Table 3). The glycosuria declined at once, and disappeared completely in 6 days. Urine nitrogen fell 1.3 gm., and the nitrogen balance rose correspondingly. A sharp rise in weight, with slower gains during the rest of the experiment, also indicated that the diet was now complete.

Ascorbic acid is not present in dried brewers' yeast, and is not re-

quired by the normal dog. We studied it as a therapeutic agent, for Dienst (1939) stated that it was beneficial in human diabetics in whom there was no dietary or clinical evidence of ascorbic acid deficiency. To determine which of the 8 vitamins might be superfluous,

TABLE 2. ATTEMPTS TO REPRODUCE THE OBSERVED EFFECT OF YEAST WITH KNOWN VITAMINS

(The Animal and the Basal Diet Were the Same as in Table 1)

Date	Weight	Nitrogen intake	Urine nitrogen	Nitrogen balance	Urine sugar	Diet and remarks
1944	kg.	gm. per day	gm. per day	gm. per day	gm. per day	
1/23–25	14.6	10.88	9.60	0.28	16.3	7 gm. yeast daily since 12/30/43, yeast replaced on 1/29/44 by 0.21 mg. thiamine, 0.42 mg. riboflavin, and 2.1 mg. nicotinic acid daily
1/26–28	14.7	10.88	9.86	−0.15	11.1	
1/29–31	14.8	10.83	10.30	−0.22	10.9	
2/ 1–3	14.8	10.83	9.90	−0.12	2.1	
2/ 4–6	14.8	10.83	8.73	1.67	4.6	
2/ 7–9	14.8	10.83	10.19	−0.29	11.8	
2/10–12	14.7	10.83	10.64	−0.61	26.8	
2/13–15	14.6	10.92	10.85	−0.70	48.7	
2/16–18	14.6	11.04	11.08	−1.03	54.4	
2/19–21	14.5	10.90	10.89	−1.65	57.7	7 gm. yeast and 2 mg. thiamine daily 2/19 to 2/26, yeast only 2/27 to 3/1. Beginning on 3/2 yeast was discontinued and 0.84 mg. thiamine, 1.68 mg. riboflavin, and 8.4 mg. nicotinic acid were given daily
2/22–24	14.5	10.88	10.04	−0.26	34.4	
2/25–27	14.6	10.88	9.78	0.03	14.6	
2/28–3/1	14.7	10.88	9.28	0.55	4.6	
3/ 2–4	14.6	11.01	10.13	0.07	13.5	
3/ 5–7	14.8	11.01	9.23	0.85	12.5	
3/ 8–10	14.9	11.01	9.35	0.52	8.9	
3/11–13	14.9	10.90	9.06	0.75	4.6	
3/14–16	15.0	10.67	9.25	0.29	9.3	
3/17–19	14.9	10.67	9.12	0.44	12.9	
3/20–22	15.0	10.86	9.39	0.65	10.7	
3/23–25	14.9	10.67	9.56	0.18	25.3	
3/26–28	14.9	10.67	9.28	0.41	24.4	
3/29–31	15.0	10.67	9.60	−0.01	17.0	
4/ 1–3	14.9	10.67	9.89	−0.35	29.7	
4/ 4–6	15.0	10.67	9.53	0.12	32.6	
4/ 7–9	15.0	10.67	9.45	0.39	34.1	In addition to the above vitamins, 10 mg. Na pantothenate and 50 mg. ascorbic acid were fed daily after 4/16
4/10–12	15.0	10.67	9.47	−0.04	26.7	
4/13–15	14.9	10.67	9.82	−0.04	45.7	
4/16–18	14.9	10.67	9.48	0.38	40.8	
4/19–21	14.9	10.67	9.28	0.20	26.6	
4/22–24	14.9	10.67	9.74	−0.20	42.7	

we first withdrew ascorbic acid, para aminobenzoic acid, and pantothenate. Slight and transient glycosuria occurred about a month later. More pronounced but also transient glycosuria occurred after withdrawal of pyridoxin. Thiamine, riboflavin, and nicotinic acid were next omitted, leaving inositol as the only dietary supplement. Glycosuria did not develop during the next 23 days, after which the animal began to refuse food. Further experiments do not support the idea that inositol alone is the component of yeast that prevents glycosuria, but it appears to be one of the active constituents. Failure of glycosuria, and incidentally losses of weight and nitrogen, to recur in the present experiment illustrates the difficulty of determining the minimum number of factors involved.

TABLE 3. DUPLICATION OF THE EFFECTS OF YEAST, AND ATTEMPTS TO FIND THE MINIMUM NUMBER OF REQUIRED FACTORS

Date	Weight	Nitrogen intake	Urine nitrogen	Nitrogen balance	Urine sugar	Diet and remarks
1944	kg.	gm. per day	gm. per day	gm. per day	gm. per day	
4/25–27	14.9	10.67	9.68	−0.04	36.6	See footnote 1.
4/28–29		10.67	9.67	0.14	35.7	
4/30–5/2	14.9	10.67	8.57	0.70	14.2	para-Aminobenzoic acid, pyridoxin, and inositol daily, beginning 4/30. Pantothenate, ascorbic acid, and para-amino benzoic acid discontinued 5/9.
5/ 3–5	15.1	10.67	8.37	1.07	4.2	
5/ 6–8	15.4	10.67	8.39	1.04	0	
5/ 9–11	15.4	10.67	8.58	0.91	0	
5/12–14	15.5	10.64	8.73	0.82	0	
5/15–17	15.5	10.63	9.36	0.18	0	
5/18–20	15.5	10.63	8.79	0.76	0	
5/21–23	15.5	10.63	8.92	0.62	1.5	
5/24–26	15.6	10.63	9.36	0.20	0	
5/27–29	15.5	10.63	8.88	0.87	2.3	
5/30–6/1	15.6	10.63				See footnote 2.
6/ 2–4	15.5	10.63	8.76	0.87	10.3	
6/ 5–7	15.5	10.63	8.88	0.66	13.5	
6/ 8–10	15.5	10.63	8.88	0.65	14.1	
6/11–13	15.6	10.63	8.71	0.98	5.8	
6/14–16	15.7	10.63	8.76	0.98	3.2	Pyridoxin discontinued on 6/14, leaving thiamine, riboflavin, nicotinic acid, and inositol
6/17–19	15.7	10.63	8.88	0.87	14.4	
6/20–22	15.7	10.69	9.01	0.74	18.5	
6/23–25	15.7	10.69	9.18	0.57	19.8	
6/26–28	15.9	10.72	8.95	0.91	2.0	
6/29–7/1	15.8	10.90	9.49	0.56	14.2	
7/ 2–4	15.9	10.90	9.03	0.85	4.7	
7/ 5–7	15.9	10.90	9.68	0.21	3.4	
7/ 8–10	15.9	10.90	10.27		6.6	
7/11–13	16.0	10.90	9.23	0.65	0.7	Discontinued thiamine, riboflavin, and nicotinic acid on 7/11, leaving inositol
7/14–16	16.0	10.90	9.53	0.35	0	
7/17–19	16.0	10.90	9.80	0.13	2.2	
7/20–22	16.0	10.90	9.47	0.46	3.1	
7/23–25	16.1	10.90			1.1	
7/26–28	16.1	10.90	9.40	0.57	4.9	
7/29–31	16.1	10.90	9.10	0.93	5.1	
8/ 1–3						Ate irregularly

[1] Thiamine, riboflavin, nicotinic acid, sodium pantothenate, and ascorbic acid were present in the diet since April 16 (Table 2). The doses of these and other factors are given in the footnote to Table 4.

[2] Accidental omission of the morning dose of insulin on June 1 resulted in excretion of 57.5 gm. of glucose that day, and loss of nitrogen. Stools for the period of July 8 to 10, and part of the urine on July 24, were lost.

Blood Sugar, Ketosis, and Lipemia

Since the usual type of sugar tolerance test could not be done in experiments which required a constant diet, we determined the blood sugar before, and 1, 2, and 5 hours after the morning meal and insulin. The results are shown in Table 4. The data for Dog 33 were secured during experiments shown in the three preceding tables. Glycosuria occurring in this animal during dietary deficiency was accompanied by an increase and prolongation of the hyperglycemia which followed the meal. When glycosuria was abolished by feeding all 8 of the dietary factors considered, the blood sugar curve was also normal.

In the experiment on Dog 34, shown in Table 4, glycosuria, hy-

perglycemia, and diminished sugar tolerance were again produced by withdrawing yeast from the diet. We next determined whether or not the glycosuria could be controlled with insulin. It was possible to do this by increasing the dose of insulin 50 per cent. The weight of the animal continued to rise, from 14.2 kg. on February 24 to 15.9 kg. on April 15. After eating the yeastless diet for 52 days, the dog began to refuse some of it, and feeding of yeast was resumed.

TABLE 4. AN INCREASE IN POSTABSORPTIVE AND POSTPRANDIAL BLOOD SUGAR VALUES ACCOMPANIES GLYCOSURIA RESULTING FROM DIETARY DEFICIENCY

Dog	Date	Factors* supplementing basal diet	Total insulin daily	True sugar per 100 ml. of blood				Urine sugar on day of test
				Before meal	Hours after meal 1	2	5	
	1944		units	mg.	mg.	mg.	mg.	gm.
33	3-11	1, 2, 3 for 10 days	14	130	184	152	164	3.2
	3-23	Same for 22 days	14	170	260	256	232	16.3
	4- 1	Same for 31 days	14	272	318	258	250	26.7
	4 15	Same for 45 days	14	278	322	304	268	48.8
33	5- 6	All 8 factors	14	98	194	174	82	0
	5-23	1, 2, 3, 4, 5	14	58	102	86	168	0
	5-29	Same, 6 days later	14	152	136	136	264	3.0
	6- 9	Same, 17 days later	14	150	256	254	280	19.7
34	2-22	Yeast	22	190	304	190	156	10.0
	3- 9	None for 15 days	22	238	278	218	190	30.0
	3-15	None for 21 days	24	278	336	284	238	23.7
	3-24	None for 30 days	28	346	318	260	238	16.7
	3-30	None for 36 days	33	174	184	120	94	0
	4-15	None for 52 days	33	154	272	72	76	0
35	3-23	None for 3 days	21	104	90	78	204	2.3
	3-30	None for 10 days	21	96	60	50	130	0
	4- 6	None for 17 days	21	114	108	108	114	0

* Numbers represent the following factors and daily doses:
1. Thiamine hydrochloride, 0.84 mg.
2. Riboflavin, 1.68 mg.
3. Nicotinic acid, 8.4 mg.
4. Inositol, 40 mg.
5. Pyridoxin hydrochloride, 2 mg.
6. para-Aminobenzoic acid, 100 mg.
7. Sodium pantothenate, 10 mg.
8. Ascorbic acid, 50 mg.

In the last experiment, on Dog. 35 (Table 4), withdrawal of yeast from the diet produced neither glycosuria nor a rise of the blood sugar curve in 17 days. Like Dog 34, this animal received 200 gm. of the diet, containing however vitamin-free casein (Labco). Even so, we were never able to produce glycosuria by withdrawing yeast from the diet of this animal. Anorexia appeared without development of glycosuria, and even when appearance of anorexia was delayed by supplying thiamine alone no glycosuria occurred. Coprophagia was carefully watched for, but never observed, Since intestinal bacteria might be important, the effect of succinyl sulfathiazole is being studied in this animal.

Tests with nitroprusside by the Rothera technique indicated that glycosuria in these animals was unaccompanied by ketosis even when

XUM

more than 50 gm. of sugar per day was being excreted. It is perhaps more remarkable that Martin (1937) did not produce ketonuria in his animals.

The total fat of the blood plasma was determined in postabsorptive specimens during the experiments in Table 1. When yeast was withdrawn on November 1, the values rose from the control level below 700 mg. per 100 ml. of plasma to 1161 mg. and 1154 mg. on November 11 and 13, but immediately fell again. After the second withdrawal of yeast no values above 796 mg. were obtained. There is thus no evident similarity between effects of B-group deficiency and effects of anterior pituitary preparations in similar experiments (Gaebler and Galbraith, 1941), although glycosuria and nitrogen loss occur in both instances.

DISCUSSION

The glycosuria which followed withdrawal of yeast from the diet in these experiments can not be ascribed to the increase in protein metabolism, for this was much too small to account for the amount of glucose excreted. Moreover, in the experiment in Table 2, replacement of yeast, on March 2, with adequate amounts of thiamine, riboflavin, and nicotinic acid almost completely prevented a rise in urine nitrogen, but the glycosuria still developed.

When glucose excretion reached its maximum, it approximately equalled the amount of carbohydrate present as such in the diet. One naturally suspects that the glucose which originated rapidly from digestion of starch was not conserved in the normal manner by conversion to glycogen or fat, and was therefore lost. If the glycosuria were due to lack of enzymes derived from dietary factors and specifically required in the oxidative process, one would not expect to control it for a long time by supplying more insulin.

SUMMARY

Experiments were performed on 3 depancreatized dogs receiving a constant diet of casein, cracker meal, corn oil, calcium phosphate, salt mixture, and haliver oil. Pancreatic enzymes and lipocaic were supplied by feeding 15 gm. of pancreatin daily. The diet was completed by adding yeast. In 2 animals the minimal dose of insulin required to control glycosuria when the diet was complete became inadequate when yeast was removed from the diet. The glycosuria which developed was accompanied by an increase and prolongation of the alimentary hyperglycemia that followed meals. The daily output of glucose reached 50 to 70 gm., and about equalled the amounf of carbohydrate present as such in the diet. Lipemia and ketosis were negligible, but losses of weight and nitrogen occurred. Resumption ot yeast feeding abolished the glycosuria in 12 days, and restored nitrogen equilibrium, with the insulin dose constant. In 1 animal it was also

shown that the glycosuria could be controlled by increasing the dose of insulin 50 per cent.

The effects of yeast could be duplicated by feeding 7 of its known constituents: thiamine, riboflavin, nicotinic acid, inositol, pyridoxin, pantothenic acid, and para aminobenzoic acid. It was therefore unnecessary to assume that an unknown constituent, such as glucokinin, can be absorbed from the intestine. Thiamine, riboflavin, and nicotinic acid, given together, prevented loss of nitrogen almost completely, but only delayed the onset of glycosuria. There was no evidence that depancreatized dogs derive benefit from an excess of ascorbic acid.

In 1 of the 3 animals it was impossible to demonstrate any relationship between water soluble vitamins and insulin requirement.

REFERENCES

Burack, E., and G. R. Cowgill: *J. Biol. Chem.* 96: 685. 1932.
Collip, J. B.: *J. Biol. Chem.* 56: 513. 1923; 57: 65. 1923.
Cowgill, G. R.: *J. Biol. Chem.* 56: 725. 1923.
Dienst, C.: *Deutsche med. Wchnschr.* 65: 710. 1939.
Gaebler, O. H., and H. W. Galbraith: *Endocrinology* 28: 171. 1941.
Gaebler, O. H., and S. M. Tarnowski: *Endocrinology* 33: 317. 1943.
Lusk, G.: The Elements of the Science of Nutrition, Philadelphia and London. 4th. Edition: 682. 1931.
Martin, R. W.: *Ztschr. f. physiol. Chem.* 248: 242. 1937.
Mattill, H. A.: *J. Biol. Chem.* 55: 717. 1923.
Nasset, E. S., Pierce, H. B., and J. R. Murlin: *J. Lab. & Clin. Med.* 16: 1151. 1931.

XUM

NOTES AND COMMENTS

FAILURE OF THIOUREA TO ALTER THE AUTONOMIC RESPONSES OF INTACT ANIMALS

THE WIDESPREAD USE of thiourea and thiourea like compounds in experimental physiology and therapeutics makes it imperative that every phase of their pharmacological activity be elucidated. It has been demonstrated (Friedenwald and Buschke, 1943) that thiourea introduced into the glucose-tyrode bath of the isolated rabbit gut in concentrations of 10^{-3} to 6×10^{-3} molar will prevent the inhibitory effect of small doses of epinephrine (2 to 10 μg./L) on intestinal movements. Similarly thiourea will prevent the stimulatory effects of epinephrine on the rabbit uterus. In their analysis of this antagonistic effect of thiourea to epinephrine, Friedenwald and Buschke compared thiourea to the cyanide ion which had very similar effects. The results could therefore be explained on the basis of metal binding and paralysis of heavy metal catalysis which is considered by most observers to be part of the mechanism by which epinephrine action is initiated. We have been able to confirm their experiments in our laboratory. However, in view of the fact that all these experiments were made on isolated organs, it was decided to determine if they could be duplicated on the intact animal and its possible effects on certain other autonomic functions as well.

EXPERIMENTAL

Cats anesthetized with cloralose (100 mg/kg) were prepared for the recording of stomach and intestinal movements (Babkin, 1928) and blood pressure. In a few cats the duct of the submaxillary gland was cannulated and control readings of the rate of salivary secretion upon stimulation of the peripheral ends of the cut chorda tympani and cervical sympathetic trunk were made. Control readings were established for the depressant effects of stimulation of the peripheral ends of the cut vagus on the blood pressure and pulse and excitory effects on the stomach and intestine. Controls were also established for the stimulatory effects of 5 μg/kg of epinephrine on blood pressure and the inhibitory effects on the stomach and intestine. In one experiment thiourea in doses ranging from 60 mg. to 200 mg./kg. were given intravenously and then either the vagus was stimulated or the 5 μg/kg. dose of epinephrine was given intravenously. Comparison with control records demonstrated that these doses of thiourea had absolutely no effect on either vagus stimulation or the intravenous injection of epinephrine on stomach and intestinal movements or on blood pressure or pulse. In this experiment also the rate of salivary secretion of the submaxillary gland with stimulation of the sympathetic trunk and the chorda tympani was measured directly after the injection of thiourea. No change in the rate from the control level was found.

In another preparation a continuous infusion of epinephrine at the rate of 3 μg./kg. per minute was given. This naturally caused a new level of blood pressure and pulse to be established. Stomach and intestinal movements were inhibited. Intravenous injection of thiourea at this point in dosages

Received for publication November 22, 1944.

ranging from 10 to 200 mg./kg. caused no change in the blood pressure curve or intestinal movements. In the final preparation thiourea was infused intravenously at the rate of 1 cc. per minute of a 5 molar solution. No change was noted in pulse, blood pressure or stomach and intestinal movements. Vagus stimulation and injection of 5 μg./kg. of epinephrine did not produce results different from the control levels established for this preparation.

Eight experiments on intact rats anesthetized with chloralose (90 mg./kg.) in which stomach and intestinal movements only were recorded were also made. Doses of thiourea ranged from 10 mg. to 200 mg./kg. In none could any deviation from the control effects of vagus stimulation and epinephrine injection be found.

In order to test the results after prolonged feeding by mouth, three rats were placed on a diet of ground dog chow with 2% thiouracil. They were sacrificed on the seventh, fifteenth, and twenty-first day of feeding respectively. Experiments on vagus stimulation and epinephrine injection on intestinal movements were normal.

A 5 molar solution of thiourea was instilled in the eye of a rabbit and cat and allowed to rest for thirty minutes. Then the peripheral end of the cervical sympathetic was stimulated on the same side and it was found that dilation of the pupil was not changed; the opposite untreated eye on sympathetic stimulation gave indentical pupillary responses. Therefore, from these results it must be concluded that in vivo thiourea has no effect on the autonomic functions described.

COMMENT

Why is it that the antagonistic effect of thiourea can be demonstrated in vitro and not in vivo? Several reasons appear probable. It must be noted that in vitro the effective dosage range of thiourea is small, not more than 10^{-4} to 6×10^{-3} molar. It is very difficult to duplicate this concentration in vivo. Also in vitro the effects are evanescent. Epinephrine must be introduced into the glucose-tyrode bath within a few seconds after the thiourea, otherwise the antagonistic effect is not noted. Again in vivo it is difficult to duplicate this time relationship. It must also be noted that only small doses of epinephrine are counteracted in vitro. Finally in vivo there is the added presence of both red blood cells and plasma in the reaction. Addition of either of these to the glucose-tyrode bath of the isolated gut or uterus prevented the antagonistic effects of thiourea to epinephrine. It may be that the phenomenon cited by Friedenwald and Buschke and confirmed by us in vitro are purely bath effects and not applicable to the intact animal.

SUMMARY

The results of Friedenwald and Buschke (1943) on the antagonistic effect of thiourea in concentrations of 10^{-3} to 6×10^{-3} to epinephrine in dosages of 2 to 10 μg./L in the glucose-tyrode bath of the isolated gut and uterus of the rabbit were confirmed by us. Attempts to duplicate these results in the intact cat and rat were unsuccessful as were the results on rats fed thiouracil from seven to twenty-one days. Experiments on the pupillary reaction to sympathetic stimulation, to salivary secretion and to vagus stimulation on intestinal movements and blood pressure and pulse after doses intravenously of from 10 to 200 mg. of thiourea per kilogram in rats and cats were also entirely negative. It is therefore concluded that thiourea,

XUM

in the dosages used, has no effect on autonomic mechanisms in the intact animal.

JOSEPH R. DIPALMA AND NICHOLAS B. DREYER
Department of Physiology and Pharmacology,
Long Island College of Medicine

REFERENCES

BABKIN, B. P.: *Die Aussere Sekretion der Verdauungsdrüsen*, 2nd Edition, p. 845, 1928.
FRIEDENWALD, J. S., AND W. BUSCHKE: *Am. J. Physiol.* 140: 367. 1943.

THE EFFECT OF STRAIN, ESTROGEN, AND DOSAGE ON THE REACTION OF THE RAT'S PITUITARY AND ADRENAL TO ESTROGENIC STIMULATION

There have been many reports that prolonged treatment of rats with estrogenic hormones frequently leads to the production of chromophobe adenomata of the pituitary (Zondek, 1936; Wolfe and Wright, 1938; Nelson, 1943; Selye, 1944). Unfortunately, most of the workers employed non-pedigreed rats or failed to specify the strain of rats used. The situation in the mouse is somewhat different. There exist marked strain differences in the occurrence of hypophyseal chromophobe adenomata following estrogenic stimulation (Gardner and Strong, 1940; Gardner, 1941).

The literature is replete with reports that administration of diethylstilbestrol often leads to an hypertrophy of the adrenal cortex (Noble, 1938; Mellish et al., 1940; Von Hamm et al., 1941). It has been suggested that the effect of diethylstilbestrol on carbohydrate metabolism is mediated through the adrenal cortex (Janes and Nelson, 1942), but Ingle (1943, 1944) believes that the effect is, in part at least, extra adrenal in nature. Furthermore, uncertainty exists as to whether the administration of naturally occurring estrogens regularly leads to hypertrophy of the adrenal cortex (Ellison and Burch, 1936; Lausen et al., 1937).

In the course of an investigation on estrogen-induced mammary carcinoma in inbred strains of rats, it was observed that the pituitary and adrenal weights differed markedly in the treated animals of the various strains. Accordingly, an investigation of the effect of estrogens on pituitary and adrenal weights was undertaken.

METHODS

Groups of adult castrated male rats of the Fischer and AXC inbred strains were injected daily for 56 days with various dosages of estrogens and/or testosterone propionate. All injections were made subcutaneously in 0.1 cc. of sesame oil. At the end of the injection period the animals were killed with ether and the pituitaries and adrenals dissected free of extraneous tissue and weighed on a microtorsion balance.

RESULTS

The results are presented in Table I. Castration increased the weight of the pituitary. This increase in weight was prevented by the daily adminis-

Received for publication January 8, 1945.

TABLE I

Group	Number of rats		Dosage	Treatment	Pituitary weight in mg.		Adrenal weight in mg.	
					MEAN±P.E.	S.D.±P.E.	MEAN±P.E.	S.D.±P.E.
Fischer males	10	intact	Controls		9.2±0.27	1.26±0.19	39.8±1.45	6.78±1.02
Fischer males	11	castrated	1 mg.	Diethylstilbestrol	84.4±4.47	21.97±3.16	82.6±0.96	4.70±0.68
Fischer males	8	castrated	250 μg.	Diethylstilbestrol	57.8±3.47	14.55±2.45	80.3±2.74	11.51±1.94
Fischer males	10	castrated	50 μg.	Diethylstilbestrol	46.0±1.90	8.89±1.34	69.6±1.30	6.08±0.92
Fischer males	10	castrated	10 μg.	Diethylstilbestrol	19.7±0.72	3.38±0.51	57.6±1.18	5.52±0.83
Fischer males	10	castrated	50 μg.	Estrone	16.7±1.00	4.67±0.70	49.9±1.36	6.37±0.96
Fischer males	10	castrated	250 μg.	α-Estradiol	73.1±3.03	14.19±2.14	45.9±0.94	4.43±0.67
Fischer males	10	castrated	50 μg.	α-Estradiol	42.7±1.84	8.62±1.30	38.8±1.22	5.70±0.86
Fischer males	10	castrated	0.5 mg.	Testosterone Propionate	9.2±0.12	0.50±0.08	34.9±0.49	2.04±0.34
Fischer males	8	castrated	0.5 mg.	Testosterone Propionate +50 μg. α-Estradiol	11.0±0.23	1.07±0.16	41.7±1.12	5.25±0.79
Fischer males	9	castrated	Controls		12.1±0.37	1.66±0.26	37.9±1.35	6.01±0.96
AXC males	13	castrated	1 mg.	Diethylstilbestrol	34.0±1.77	9.45±1.25	48.5±1.22	6.52±0.86
AXC males	8	castrated	250 μg.	Diethylstilbestrol	35.5±2.21	9.26±1.56	42.9±0.79	3.33±0.56

tration of 0.5 mg. of testosterone propionate, an amount sufficient to produce sex accessories of approximately twice the weight of those of normal intact males.

All of the estrogens in the dosages employed produced significant increases in pituitary weight although diethylstilbestrol and α-estradiol were more active in this regard than estrone. The increase in pituitary size which followed the administration of 50 μg. of α-estradiol daily was prevented by the simultaneous administration of 0.5 mg. of testosterone propionate. In the Fischer rats the pituitary response was proportionate to the dosage of estrogen administered. In rats of the AXC strain on the other hand, both dosages of diethylstilbestrol produced similar increases in pituitary weight.

The only compound that produced a marked increase in the adrenal weights of the Fischer rats was diethylstilbestrol. All of the dosages of diethylstilbestrol which were employed produced a significant increase in adrenal weight, the maximum increase being obtained with 250 μg. daily. A much smaller increase was produced in the adrenals of the AXC strain. A slight increase in the adrenal weights of the Fischer strain occurred with 250 μg. of α-estradiol while a somewhat greater increase was obtained with 50 μg. of estrone.

DISCUSSION

From these results with the various estrogens and two inbred strains of rats an explanation of the divergent results that have been reported in the literature is apparent. The pituitary response in the two strains is vastly different, i.e., 50 μg. daily of diethylstilbestrol in the Fischer strain produces larger pituitaries than 1 mg. daily in the AXC strain. Diethylstilbestrol has the most marked effect on both the adrenal and pituitary weights. Estrone is less effective than α-estradiol in the production of pituitary enlargement but is more effective in increasing the adrenal weights.

SUMMARY

Diethylstilbestrol produces marked pituitary enlargement in the inbred Fischer strain of rats; the enlargement is proportionate to the dosage employed. Significantly less pituitary enlargement is produced in the inbred AXC strain by diethylstilbestrol. In the Fischer strain α-estradiol is about as effective in the production of pituitary enlargement as is diethylstilbestrol, but estrone is much less effective.

The enlargement of the pituitary that is produced in the Fischer strain

by 50 μg. of α-estradiol daily can be prevented by the concomitant administration of 0.5 mg. of testosterone propionate daily.

The two strains differ markedly in adrenal response to diethylstilbestrol, the adrenal weight response of the Fischer strain being greater than that of the AXC strain. 250 μg. of diethylstilbestrol produced the maximum adrenal response in the Fischer strain. The effect of estrone and α-estradiol upon the Fischer's adrenal is less marked.

We would like to thank: Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Incorporated for α-estradiol and testosterone propionate; Dr. D. F. Robertson of Merck & Company for diethylstilbestrol and Drs. Oliver Kamm and D. A. McGinty of Parke Davis & Company for estrone. We would also like to thank Dr. W. O. Nelson for generously permitting the use of his laboratory.

ALBERT SEGALOFF[1,2] AND WILHELMINA F. DUNNING
From the Departments of Anatomy and Pathology,
Wayne University College of Medicine, Detroit,
Michigan.

REFERENCES

ELLISON, E. S., AND J. C. BURCH: *Endocrinology* 20: 746. 1936.
GARDNER, W. U., AND L. C. STRONG: *Yale J. Biol. & Med.* 12: 544. 1940.
GARDNER, W. U.: *Cancer Research* 1: 345. 1941.
INGLE, D. J.: *Amer. J. Physiol.* 138: 577. 1943.
INGLE, D. J.: *Endocrinology* 34: 361. 1944.
JANES, R. G., AND W. O. NELSON: *Am. J. Physiol.* 137: 557. 1942.
LAUSON, H., C. G. HELLER AND E. L. SEVRINGHAUS: *Endocrinology* 21: 735. 1937.
MELLISH, C. H., S. J. BAER AND A. C. MACIAS: *Endocrinology* 26: 273. 1940.
NELSON, W. O.: *Cancer Research* 2: 728. 1943.
NOBLE, R. L.: *J. Physiol.* 94: 177. 1938.
SELYE, H.: *Cancer Research* 4: 349. 1944.
VON HAMM, E., A. HAMMEL, T. E. RARDIN AND R. H. SCHOENE: *Endocrinology* 28: 263. 1941.
WOLFE, J. M., AND A. W. WRIGHT: *Endocrinolgy* 23: 200. 1938.
ZONDEK, B.: *Lancet* 1: 776. 1936.

[1] This work was completed under the tenure of a Ciba Fellowship in Endocrinology.
[2] Present address: Department of Medicine, Vanderbilt University School of Medicine, Nashville 4, Tennessee.

THE BASAL METABOLIC RATE IN MOLTING AND LAYING HENS[1]

Molting is associated with low laying activity or complete cessation of ovulation, a fact which is of both endocrinological and economical interest. It is well known that thyrotropic hormone and thyroid hormone promote molting (Torrey and Horning, 1922; Walker, 1925; Zawadowsky, 1927, 1932; Greenwood and Blyth, 1929; Mitschell, 1929; Noether, 1931, 1932; Hill and Parkes, 1935; Parkes, 1937) and impair egg laying (Zawadowsky, 1927, 1932, 1937; Zawadowsky and Rochlin, 1928; Noether, 1931, 1932).

In 1939, van der Meulen reported that the regular annual molting cycle (autumn) could be interfered with by thyroid feeding without, however,

Received for publication January 20, 1945.
[1] Because of the difficulties of international communication the authors have not read proof of this article.

affecting the laying cycle. He pointed out that this observation is in contradiction of the prevailing view and of the two existing theories of Zawadowsky (1927) and Greenwood (1936) concerning the control of the annual cycle in hens. Both theories assume that the alternate molting and laying periods are stimulated by the same mechanism. Rhythmic fluctuations in the excretion of thyrotropic hormone and gonadotropic hormone of the anterior pituitary lobe produce the molting cycle, their rate of incretion being controlled by light and temperature, i.e. environmental climatic factors (Heape's hypothesis, 1905). This would imply a possible antagonism between the thyrotropic and gonadotropic factors, an assumption made plausible by the experiments of Evans and Simpson (1930); van Horn (1931); Aron and Benoit (1931); Leonard (1936). Van der Meulen (1939) promotes a third theory which assumes that fluctuations of the incretion of thyrotropic and gonadotropic hormones induce molting and impair egg laying, both mechanisms being quite independent of each other. It is possible, but not necessarily so, that they may affect each other in a secondary way.

At the Seventh World's Poultry Congress in Cleveland, Ohio, 1939, van der Meulen stated: "We must accept the assumption that the annual molt is caused by increased thyroid activity of the birds, although it is desirable that this assumption should be supported by further experiments." In the literature accessible to us we could not find any reference to measurements of the BMR in molting hens, a fact of primary importance in order to prove a thyrogen theory of molting. The reason for the lack of data on this point seems to be due to the difficulties encountered in obtaining consistent measurements of the BMR in hens. Winchester (1940) has shown that with a closed circuit respiration apparatus of the Regnault-Reisig type, fluctuations of the BMR reaching as high as 40% of the maximum were recorded in resting hens. Our experiments partly confirm this difficulty.

EXPERIMENTAL

Determinations of BMR were carried out on a total of 40 white leghorn hens during various seasons of the year. Every hen was tested about 10 times, 8 hens were tested before, during and after molt. In the beginning we used the open system of Abderhalden (1922), in which dried CO_2-free air was drawn through a chamber containing the hen, maintained at a temperature of 28° C. The expired gases were passed through drying U-tubes and later through soda lime-NaOH tubes and sulfuric acid tubes to remove the water. This rather cumbersome method measured the CO_2 output only and gave such unreliable results that we soon abandoned it. In addition, the hens were disturbed in their laying activity. A more convenient method was found to be the simple device proposed by Williams, Phelps and Purch (1941) for guinea pigs. Here the animal sits in an atmosphere rich in O_2, the consumption of which is checked by a manometer containing Brodie-fluid. O_2 consumption is read in cc. of water which replace the air from a 1000 cc. burette and the calculation is made later for cc. of O_2 consumed per kg. animal in 1 hour at standard temperature and pressure (0° C.–760 mm. Hg). CO_2 is not measured in this procedure since it is immediately absorbed by a soda lime-NaOH mixture within the metabolism chamber. The hens were starved for 18 hours before every test which lasted 30 minutes. Molting was assessed, as usual, by counting the feathers of the tail and the wings and from negative egg-laying records.

TABLE I. O_2 CONSUMPTION OF 10 LAYING HENS DURING THE SUMMER

Hen	Laying record	Body weight	Number of tests	Average O_2 consumption per kg/h
No.	weekly	kg.		cc.
1	3	1.500	10	389 (±56)
2	3	1.475	11	410 (±64)
3	3	1.300	10	452 (±88)
4	4	1.350	10	515 (±45)
5	4	1.250	9	480 (±81)
6	4	1.400	10	390 (±72)
7	4	1.450	10	401 (±40)
8	5	1.375	10	476 (±71)
9	5	1.300	10	395 (±65)
10	5	1.200	8	498 (±85)

TABLE II. O_2 CONSUMPTION OF 10 LAYING HENS DURING THE WINTER

Hen	Laying record	Body weight	Number of tests	Average O_2 consumption per kg./h
No.	weekly	kg.		cc.
11	3	1.225	11	369 (±54)
12	3	1.250	8	410 (±75)
13	3	1.500	10	515 (±43)
14	3	1.425	10	467 (±72)
15	4	1.350	10	458 (±89)
16	4	1.275	9	491 (±80)
17	4	1.325	10	432 (±63)
18	4	1.450	10	445 (±58)
19	5	1.475	10	399 (±62)
20	5	1.300	10	501 (±51)

TABLE III. O_2 CONSUMPTION OF 8 HENS DURING LAYING AND MOLTING PERIODS

Hen	Average body weight	Summer laying period average O_2 consumption per kg./h	Autumn molting period average O_2 consumption per kg./h	Winter laying period average O_2 consumption per kg./h
No.	kg.	cc.	cc.	cc.
21	1.475	398	597	412
22	1.400	412	635	432
23	1.250	400	600	417
24	1.375	475	760	434
25	1.425	498	626	510
26	1.500	515	748	470
27	1.475	473	745	516
28	1.325	509	619	397

RESULTS

The results of our examinations of the BMR of 10 laying hens during the summer and of 10 laying hens during the winter are compiled in Tables I and II; the BMR averaging between 400 and 500 cc O_2 per kg/h. Table III, which compares the BMR of 8 laying hens during the summer with that during autumn molting and after the resumption of egg laying in the winter, deserves special attention. This table demonstrates an increase of the BMR to values averaging between 600–700 cc O_2 per kg/h during molt as compared to the lower values (400–500 O_2 per kg/h) obtained before and after molting.

We attempted to change the BMR and molting by peroral administration of small doses of Lugol's solution and diethylstilbestrol. Our results were, however, discouraging.

SUMMARY

The BMR was studied in 20 normal laying leghorn hens in summer and winter. It averaged 400–500 cc O_2 per kg/h. In 8 leghorn hens, the BMR averaged 400–500 cc O_2 per kg/h before and after molting, i.e. during egg laying periods, and increased to 600–700 cc O_2 per kg/h during molt. An attempt to check or accelerate molting by Lugol's solution or diethylstilbestrol, administered per os, failed.

We wish to thank Prof. B. Zondek under whose supervision this work was carried out. We are indebted to Miss J. Hammer and Mr. I. Tamari for their devoted help.

M. Perek[2] and F. Sulman

From the Hormone Research Laboratory of the Hebrew University, Jerusalem, Palestine

REFERENCES

Abderhalden, E.: Physiol. Practicum 3rd ed. J. Springer, Berlin, p. 163. 1922.
Aron, M., and J. Benoit: *Compt. rend. Soc. de Biol.* 108: 784, 786. 1931.
Evans, H. M., and M. E. Simpson: *Anat. Rec.* 45: 215. 1930.
Greenwood, A. W.: Berl. Welt-Geflugel Congr. Leipzig, I, 265. 1936.
Greenwood, A. W., and J. S. S. Blyth: *Proc. Roy. Soc. Edinburgh* 49: 313. 1929.
Heape, M.: *Proc. Roy. Soc. London* s.B. 76: 260. 1905.
Hill, R. T., and A. S. Parkes: *Proc. Roy. Soc. London s.B.* 117: 202. 1935.
van Horn, W. M.: *Anat. Rec.* 51: 38. 1931.
Leonard, S. L.: *Proc. Soc. Exper. Biol. & Med.* 34: 599. 1936.
Mitschell, J.: *Physiol. Zool.* 2: 411. 1929.
Noether, P.: *Naunyn-Schmiedeberg's Arch. exp. Path. & Pharm.* 160: 369. 1931; *Klin. Wschr.* 11: 1702. 1932.
Parkes, A. S.: *Biochem. J.* 31: 579. 1937.
Torrey, H. B., and B. Horning: *Proc. Soc. Exper. Biol. & Med.* 19: 275. 1922.
Van der Meulen, J. B.: 7th World's Poultry Congr. Proc., Waverly Press, Baltimore, p. 109. 1939.
Walker, A. T.: *Am. J. Physiol.* 74: 249. 1925.
Williams, C., D. Phelps and J. C. Burch: *Endocrinology* 29: 373. 1941.
Winchester, C. F.: *Poultry Sci.* 19: 233. 1940.
Zawadowsky, B. M.: *Roux Arch. f. Entwicklungsmech.* 110: 149. 1927.
Zawadowsky, B. M.: *Endokrinologie* 10: 30. 1932.
Zawadowsky, B. M.: *Bull. biol. med. exper. URSS.* 3: 119. 1937.
Zawadowsky, B. M., and M. Rochlin: *Roux Arch. f. Entwicklungsmech.* 113: 323. 1928.

[2] Generously aided by a grant from the Association of Poultry Breeders in Palestine.

ASSOCIATION NOTICES

ANNUAL MEETING CANCELLED

In compliance with request of Director of War Mobilization and Reconversion, James F. Byrnes, your Council was polled and voted (1) to cancel the 1945 annual meeting of the Association, (2) to continue the terms of the present officers for another year, and (3) that the Ciba and Squibb Awards be made in 1945.

CARL R. MOORE, *President*
HENRY H. TURNER, *Secretary-Treasurer*

CHANGE OF MANAGING EDITORSHIP

Owing to pressure of other duties Dr. Roy G. Hoskins who has been serving as Managing Editor of the Journal of Clinical Endocrinology since 1942 has resigned.

His successor is Dr. Kenneth Wade Thompson. The new official address of the Journal will be Dr. Thompson's office at 1101 Beacon Street, Brookline 46, Massachusetts.

Publication Committee
EARL T. ENGLE, *Chairman*
E. L. SEVRINGHAUS
WILLARD O. THOMPSON